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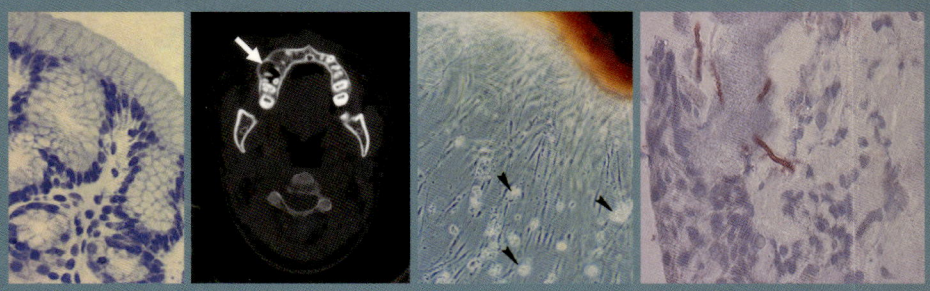

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Proceedings of the Sixth International Conference on Breath Odor

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John Greenman
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Oral Diseases

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Oral Diseases

Volume 11 Supplement 1 2005

Proceedings of the Sixth International Conference on Breath Odor

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GUEST EDITORS
John Greenman
Mel Rosenberg

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EDITORIAL

Proceedings of the Sixth International Conference on Breath Odor*

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Summary of the conference

The Sixth International Conference on Breath Odor was held at the Royal College of Surgeons of England, London on April 20th–22nd, 2004. The meeting included 70 presentations (23 oral and 47 poster) (see Appendix 1 for abstracts) from 16 countries (Belgium, Brazil, Canada, China, Denmark, Germany, Iran, Israel, Italy, Japan, South Korea, Netherlands, New Zealand, Poland, UK, and USA). The number of papers presented is the largest when compared with all five previous conferences, and the consensus was that the scientific level of the presentations was extremely high.

The scientific community dealing with breath odors is finally reaching consensus as to what are the main issues that require resolution in the coming years. This has been catalyzed by the publication of the ADA guidelines for submission of products for ADA approval that recently appeared. These guidelines, introduced by Wayne Wozniak in his presentation, were the subject of much subsequent discussion during the meeting. The publication of the guidelines is a brave, initial attempt to codify oral malodor measurement, and the ADA acknowledges that they may be modified and improved in the coming years. Thus the onus is now on the research community to suggest how to approach this new challenge.

Several of these challenges are summarized below.

1 Standardization and calibration of odor judges. In the near future, organoleptic scoring is likely to remain as the 'gold standard' method of measuring bad breath, both for the industry and academia. In his presentation, Steve Mason of Hilltop was forthcoming on Hilltop's methodologies. These can now be compared with the independent methods suggested in academic research (e.g. the Greenman Group), and a consensus can be developed in the coming years.
2 Measurement of individual gases. Volatile Sulphur Compounds (VSC) are recognized as being highly associated with oral malodor, and individually measured using the gas chromatography or combined using the Halimeter. Results using the latter may not be as robust as those obtained using GC, but it is easy to use and correlates with the odor judge scores, making it useful in industry and academia. Other instruments, based on VSC and other gases are likely to compete in the coming years, until one with an extremely high correlation with odor judges (e.g. $r > 0.8$) is developed and tested.
3 Trials. How different is morning odor from daytime odor? How do these impact on measurement methodologies? Similarly, how similar are tongue and interdental (or subgingival) odor? Can these two be indiscriminately mixed and matched in trials? What reductions and time periods are appropriate for products that reduce malodor or that prevent malodor from occurring?
4 Microorganisms and their substrates? Which microorganisms are responsible for oral malodor? Are there species which mitigate malodor production (*Streptococcus salivarius* has been suggested in one report, but has been linked to malodor production in another). How does substrate availability and location impact on malodor quality and quantity? Do challenge tests (e.g. with cysteine) reflect on the normal *in vivo* situation? Can biofilm research be helpful in our quest to understand oral malodor? How useful are enzymatic tests?
5 Can components of breath (whether odorous or not) be used to indicate oral and other medical conditions? Can this be accomplished using simple methodologies?
6 How should excessive self-assessment of bad breath be classified, measured and treated? How can people be encouraged to objectively rate their own oral malodor?
7 How should dentists, physicians and other caregivers relate to this common problem?

In addition to over 60 oral presentations and posters (many represented here), the meeting featured four breakout workshops dealing with, (i) odor measurements using human judges, (ii) odor measurements using

*Conference jointly organized by the International Society of Breath Odor Research (ISBOR) and the Eastman Dental Institute for Oral Health Care Sciences, London and held on the 20th–22nd April, 2004 at the Royal College of Surgeons of England, London.

instruments and other laboratory tests, (iii) role of microbiology, and (iv) breath odor trial design and statistics. The workshops were all well attended and the consensus reports are included here (Appendix 2).

Finally, on behalf of all the participants, we would like to express our thanks to all those from the Eastman Dental Institute who have spent much energy in preparing and organizing this conference and our sponsors. We gratefully acknowledge the generous support of the following organizations: Quest International, TheraBreath Oral Products (Platinum sponsors); Abilit Corporation, Colgate–Palmolive (Gold sponsors); GlaxoSmithKline, Bee Brand Medico Dental Company, OneDropOnly, Procter & Gamble (Silver sponsors); Quintessence, British Dental Association, Fresh Breath Ltd (minor sponsors). We are looking forward to the next (7th) Conference of ISBOR in 2 years.

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INVITED GUEST REVIEW

The ADA guidelines on oral malodor products

WT Wozniak

American Dental Association, Chicago Headquarters Building, Chicago, IL, USA

For over 130 years the ADA has been an important information source on the safety and effectiveness of dental products. The Council has recently completed the development of Acceptance Program Guidelines for products used in the management of oral malodor. The ADA Seal Program will ensure that professional and consumer dental products meet rigorous ADA criteria for safety and effectiveness
Oral Diseases (2005) 11 (Suppl. 1), 7–9

Keywords: ADI guidelines; oral malodor products

Introduction

For over 130 years, the American Dental Association (ADA) has been an important information source on the safety and effectiveness of dental products both for the consumer and the clinician. The ADA Seal of Acceptance Program has assisted the public and profession in making decisions regarding dental therapeutics, materials, instruments, and equipment by its systematic review of product evaluations and testing. There is no other professional evaluation program like it anywhere else in the world. Every day the Association receives inquiries from all over the world requesting information on products, particularly those that have been awarded the ADA Seal of Acceptance. Currently, some 1300 professional and consumer products from more than 320 dental manufacturers display the ADA Seal of Acceptance. Each year the ADA Seal Evaluation program reviews more than 200 products. Using stringent guidelines for reviewing product tests, ingredients, labeling, advertizing, promotional claims, and patient education materials, the program provides the means for the ADA to supply reliable information to the profession and consumers. Furthermore, not every submitted product qualifies for the Seal. The ADA currently rejects about 30% of products when they are initially submitted for the Seal, which attests to the program's rigorous criteria, as well as the overall need for such a program.

History

During the First World War, the USA government asked the National Bureau of Standards to establish national specifications for the purpose of ordering supplies for the military. In addition, the War Department requested a thorough laboratory and clinical investigation of the physical properties of dental amalgam. The Association agreed to participate at the National Bureau of Standards in a collaborative agreement with the government, which has resulted in written specifications for materials used in dentistry. Today, the ADA's Paffenbarger Research Center, located at what is now the National Institute of Standards and Technology, still contributes significantly to the profession's scientific research and new product development.

After World War I, the Association joined with the American Medical Association (AMA) to analyze therapeutic drugs. This collaboration led to the establishment of the Council on Dental Therapeutics (CDT) in 1930. At that time the AMA had an acceptance program that served as a model for the ADA. The ADA's role in dental product evaluation first came to the forefront during the Council's review of information on a product called Pyros. This product claimed to cure 'pyorrhea'. However, the Council found the product to be ineffective and the USA government seized the product and did not allow it to be marketed. A court battle then took place with the final ruling being that the product's assertions were fraudulent. Based on this case, the role of the ADA in evaluating safety and effectiveness of products, reviewing advertizing claims, and informing the profession and public about products was solidified. Later in that year, the CDT developed rigorous guidelines for testing dental products and procured other health care products for evaluation. The ADA Acceptance Program was born. In 1934, the CDT published Accepted Dental Remedies, which became Accepted Dental Therapeutics that was published for many years and has now been replaced by the ADA Guide to Dental Therapeutics.

While this was ongoing the specifications program was continuing at the National Bureau of Standards until 1953. At that time, the International Association for Dental Research (IADR) assumed this responsibility as an advisor to develop specifications for the profession. In 1970, the American National Standards Institute (ANSI) held a national conference and replaced the Specification Committee of the IADR with the newly created American National Standards Committee (ANSC) MD 156. In 2000, the ADA was accredited by ANSI as an Accredited Standards Organization. As a result the current ADA Standards Committee on Dental Products (SCDP) was formed. This committee is responsible for development of all dental standards in the USA. The committee is composed of eight subcommittees: Restorative Materials,

Prosthodontic Materials, Terminology, Instruments, Equipment, Oral Hygiene Products, Implants, and Products for Infection Control. These subcommittees oversee activities of some 60 working groups that are responsible for the development or revision of over 100 ANSI/ADA standards or technical reports. More than 800 volunteers participate in the development of dental standards. The ADA SCDP also considers international standards developed by the International Organization for Standardization (ISO) for adoption as ANSI/ADA specifications. In fact, approximately 80% of current ANSI/ADA standards are also ISO standards. The ADA was very instrumental in the development of ISO/Technical Committee 106, which is responsible for international dental standards. Many of the experts in the ADA SCDP also participate in the activities of ISO/TC 106.

Product evaluation

Standards thus formulated by the ADA SCDP are used in the ADA Seal of Acceptance Program for product evaluation. The ADA has been in business of product testing in its laboratories since the 1920s first at the National Bureau of Standards and since 1966 at the ADA Headquarters in Chicago. The laboratories at the ADA were recently renovated and modernized. Currently some 100 products, both consumer and professional are tested every year for compliance with ANSI/ADA specifications. As mentioned previously, many submitted products fail to meet these criteria. For example, in one recent 18-month period, of the 125 products tested, 57 failed to meet the appropriate standard. In these cases, the Association works with manufacturers to assist in correcting product deficiencies. These efforts have led to changes in labeling, product reformulations, and improvements in quality control. Eventually many of these products have received the ADA Seal.

Guidelines

The Association also evaluates products in its Acceptance Program for which ANSI/ADA standards do not exist. For these products the ADA Council on Scientific Affairs has developed Acceptance Program guidelines. ADA Acceptance Program guidelines developed from the ADA's Provisions for Acceptance, describe in detail the types of laboratory and/or clinical studies needed for Council review of the product. Typically specific procedures for evaluation of physical properties, chemical properties, biocompatibility and most importantly clinical efficacy are included in the guidelines. Currently ADA Acceptance Program Guidelines have been published for over 40 products areas including dental materials, instruments, equipment, therapeutic products, infection control products, and many consumer products such as fluoride dentifrices, toothbrushes, and dental floss.

Oral malodor

Late in 2003, the Council completed the development of Acceptance Program Guidelines for Products Used in the Management of Oral Malodor. The Association began this process because of a growing interest in the public and profession over the prevalence of oral malodor in the adult population and developments in technology and possible treatments. Estimates are that from 25 to 50% of adults suffer from persistent oral malodor and that the condition may rank only behind dental caries and periodontal diseases as the chief complaint of dental patients. Development of the guidelines began in 1998 with the writing of the first draft by the Council and consultants. This draft was submitted to interested parties in 1999 and after review of comments, revised and resubmitted for interested parties review in 2000. At that time it became clear that certain aspects of the guidelines needed further study. The Council decided that a conference should be held to address these concerns. This consensus conference on Diagnosis and Management of Oral Malodor was held at the Association in November 2001. The conference addressed many of the concerns found in the previous interested parties' reviews of the draft guidelines. These included clinical trial design, measurement methods and level of product efficacy and statistical analysis of oral malodor data. In addition, the conference helped in the drafting of a Council statement on oral malodor that was eventually published in the Journal of the ADA in February 2003. Based on the recommendations from the conference the Council revised the guidelines and presented them again for interested party comment in 2002. Additional comments were received again on four major areas: length of clinical trials, initial level of oral malodor in the clinical trials, expected degree of oral malodor reduction in the clinical trials, and number and training of the oral malodor judges. After considerable review and discussion the Council approved the guidelines for publication in late 2003.

The guidelines as published apply to products that are designed to manage oral malodor of non-systemic origin. This type of oral malodor is generated by microorganisms or metabolic compounds that reside on the teeth, tongue or other areas in the oral cavity. Such malodor corresponds to approximately 90% of the observed cases. Products that manage such malodor by either chemical agents or mechanical means may be considered under these guidelines.

Safety of oral malodor products

Regarding safety, the guidelines require that a 6-month study should be conducted unless the product has already been used for plaque and gingivitis control or whose active ingredient is generally recognized as safe. If not, oral flora should be monitored over a 6-month period in appropriately sized clinical study to determine if development of opportunistic and pathogenic organisms occurs. In addition, effects on oral soft and hard tissues should be assessed. As some chemical agents may cause an increase in pathogenic organisms, gingival inflammation should be measured with an appropriate index, e.g. Loe and Silness.

Also evidence should be provided that the product does not adversely affect soft tissues in other ways such as staining. Any evidence of other pathologic conditions such as allergic reactions, oral ulcerations, candidiasis, or secondary infections of the oral mucosa should be noted. For hard tissues and restorative materials, evidence of lack of effects such as staining, shade alterations or loss of structure should be provided. For loss of structure, surface examination may be sufficient. Assessments of possible toxic effects of the active agent or other product factors should be conducted. These should include standard toxicologic profiles, depending on the particular product. Data on the mutagenicity and the carcinogenicity of the product or its active agents must also be submitted. Any patient reports of changes in taste, changes in salivary flow, burning sensations or xerostomia should be reported. Finally, oral flora should be monitored in subjects for the development of opportunistic and pathogenic organisms. Data should be obtained at baseline, 3 weeks and 6 months. Evidence must be provided that significant detrimental shifts in a representative sample of oral flora have not occurred.

Efficacy

According to the ADA guidelines, to demonstrate efficacy in the management of oral malodor, two independent 3-week clinical studies utilizing an appropriate placebo control should be conducted. Either crossover or parallel group designs are permitted. Measurements of oral malodor should be performed at a minimum of two appropriate time periods after baseline during the 3-week period. Significant reductions in oral malodor from baseline to the subsequent timepoints relative to the placebo control should be demonstrated. In addition, 80% of the subjects should demonstrate a reduction to questionable or no oral malodor at some time during the treatment period. Furthermore, evidence should be provided from the previously discussed 6-month clinical study that development of microbial resistance does not occur. Also, mechanism of action should be given (if known).

The guidelines also give substantial information regarding the design of the clinical trials that should be conducted to demonstrate efficacy. Regarding subject selection, the guidelines recommend that individuals should be included who have intrinsic malodor of oral origin and have an average organoleptic intensity rating of at least 2.0 on a 0–5 intensity scale. Subjects with oral diseases such as advanced periodontitis and subjects who smoke or wear oral appliances should be excluded. In addition, as routine professional cleanings may reduce oral malodor, at least 1 week should elapse after a prophylaxis before participation in the study. The guidelines also recommend that measurements of oral malodor be obtained based on product claims. For example, an overnight product should be assessed at day 2 (at a minimum). Regarding oral malodor assessment, organoleptic intensity or hedonic examinations should be performed by two trained and calibrated odor judges. Judges used in the clinical trials should be calibrated using a range of standard odorants sufficient to reflect the different patterns of nose receptors. Instrumental methods may also be used to provide additional data on the level of oral malodor. For example, measurement of volatile sulfur compounds using gas chromatography or portable sulfide monitors is recommended, if available.

Other assessments and labeling

The ADA guidelines give specific information regarding other safety assessments that should be conducted on the product. For example, the microbiologic assessment is described. Also oral soft tissue assessments are needed because some chemical agents may cause an increase in pathogenic organisms. This can be performed by measuring gingival inflammation with an appropriate index such as Loe and Silness. Hard tissue effects are another area of concern. Staining and any decrease in enamel hardness should be assessed.

Finally, the Council took into account its concern about potential misuse and mistreatment by patients using oral malodor products. As part of its requirements for granting the ADA Seal the labeling for the accepted product must contain the following caution statement: 'Persistent oral malodor (bad breath) may indicate a serious underlying disease. If your bad breath persists after 3 weeks of product usage please consult your dentist or physician as soon as possible'.

Seal submission process

The ADA welcomes and encourages product submissions. Besides oral malodor products, the Council considers many professional and consumer products for the ADA Seal. Included are all therapeutic drugs and chemicals used in the diagnosis, treatment, and prevention of oral diseases. The Council also considers dental materials, instruments, and equipment that comply with the ADA's provisions, specifications, or guidelines. Analgesics, anesthetics, fluorides, cavity liners, composite resins, implants, and impression materials are just a few examples of product categories that are included in the Seal Program. Commercial products are evaluated upon the request of a distributor or manufacturer and any company may submit appropriate products to the Council for consideration for acceptance. Although the review process can be complex for some products, many products have received the ADA Seal in <90 days. There is a submission fee for consumer products but no fees are charged for professional products. Once a product is granted the ADA Seal, the acceptance period is for 3 years. Acceptance can be renewed and is usually granted if there are no changes in the product, labeling, or promotional materials.

In conclusion, the ADA Seal ensures that professional and consumer dental products meet the rigorous ADA criteria for safety and effectiveness. With the addition of oral malodor products to the ADA Seal program, consumers can now look for the ADA Seal to help them choose products that have been clearly shown to be efficacious in the treatment of intrinsic oral malodor.

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ORIGINAL ARTICLE

Trimethylaminuria (fish-odour syndrome) and oral malodour

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A small but important percentage of oral malodour cases have an extra-oral aetiology and certain of these fall into the category of 'blood-borne halitosis'. Odoriferous substances generated within the body and transported to the lungs via the circulatory system may, if sufficiently volatile, leave with the exhaled air and impart a foetid odour to the breath. The aliphatic tertiary amine, trimethylamine, is such a volatile compound that is generated to excess in patients with a metabolic disorder known as trimethylaminuria (fish-odour syndrome). This article highlights this condition and draws attention to its potential role in the causation of recalcitrant oral malodour.
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Keywords: oral malodour; trimethylamine; trimethylaminuria; 'fish-odour syndrome'

Introduction

Oral malodour (halitosis, bad breath) is a relatively common complaint that may lead to serious consequences throughout an individual's private and professional life. Determining the incidence of this condition is difficult but it has been suggested that up to half of the general population may, at sometime, exhale 'socially unacceptable breath' (Messadi, 1997; Meningaud *et al*, 1999; Sanz *et al*, 2001; Tomas *et al*, 2001). The oral cavity is the major source of this malodour, particularly the posterior dorsum of the tongue, although other associated factors such as food impaction, gingivitis, periodontal disease and oral carcinoma may play a part. However, about 10–15% of halitosis cases have an extra-oral aetiology and may be indicative of other disorders. Such causes may include problems with the respiratory tract (chronic tonsillitis and sinusitis, infection, bronchiectasis, pulmonary abscess), the gastrointestinal system (obstructions or inflammatory gastrointestinal processes) or at anatomically distant sites linked via the circulatory system (hepatic insufficiency, liver cirrhosis, renal failure, diabetes mellitus and diabetic acidosis) (Preti *et al*, 1992; Durham *et al*, 1993; Touyz, 1993; Spielman *et al*, 1996). These latter systemic diseases and metabolic disorders, together with the effects of certain medications and foods, may be grouped into the category of 'blood-borne halitosis' in which malodorous compounds in the bloodstream are transported to the lungs where they volatize and are exhaled in the breath (Tangerman, 2002).

The intensity of breath odour has shown a correlation with the presence of volatile sulphur-containing compounds, such as hydrogen sulphide, methanethiol and dimethyl sulphide, that may be produced by microbial metabolism. However, in certain cases the odour itself, or contributions to the odour, may arise from other volatile compounds that have been detected in the air expelled from the oral cavity. These candidates include short chain fatty acids (acetic, propionic, butyric), aromatics (indoles, skatole), methanol, ethanol, acetone, acetaldehyde and pentane, with the amine, cadaverine, being detected in saliva (Solis-Gaffar *et al*, 1975; Tonzetich, 1977; Tonzetich *et al*, 1991; Goldberg *et al*, 1994; Meningaud *et al*, 1999; Sanz *et al*, 2001; Volozhin *et al*, 2001). In cases of 'blood-borne halitosis' it is quite possible that virtually any odourous compound that satisfies the criteria of volatility, if generated within the body and having access to the bloodstream, could give rise to an oral malodour. Trimethylamine is such a compound. It is a simple tertiary aliphatic amine with a pungent ammoniacal odour approaching that of rotten fish at low concentrations and the human nose is extremely sensitive to this molecule, with some individuals being able to detect less than one part in 10^9 (Willey, 1985). It is produced in excess in a metabolic disorder known as trimethylaminuria or the 'fish-odour syndrome' (Table 1; Mitchell, 1996; Mitchell and Smith, 2001).

Trimethylaminuria

Biochemically, the disorder is characterized by the presence of 'greater than normal' amounts of trimethylamine within the body. These high levels are present owing to a failure in removing the amine via the usual oxidation route to the non-odourous metabolite, trimethylamine N-oxide. This situation arises from a

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Table 1 Key events in the clinical history of the trimethylaminuria (fish-odour syndrome)

Period	*Clinical observation*
1840–1860	Two isolated reports in the medical literature, suggestive of the condition
1970	First clinical description linking condition with Turner's Syndrome
	First use of the term, 'fish odour syndrome'
1970–1985	Single cases reported sporadically and concealed within world literature
1980	Recognition of a breed of domestic fowl deficient in trimethylamine N-oxidation and its relationship to the 'tainted egg syndrome'
1980–1990	Characterization of the human genetic polymorphism of trimethylamine N-oxidation.
	Recognition of the condition as a separate phenomenon
1990s	Molecular genetic basis of condition established
	Flavin monooxygenase enzyme (FMO3) mutations identified as causation
2000	Realisation of sub-types of the syndrome leading to a new classification
	Multiple factors involved in the overall causation of the condition

For more details on historical aspects see references (Mitchell, 1996; Mitchell and Smith, 2001).

mismatch in the enzyme's capacity to undertake this metabolic reaction and the substrate load it has to process (Mitchell, 1996; Mitchell and Smith, 2001). Consequently, in principle, it appears that there are two major sub-types of the condition. Firstly, there are those forms that are related to a dysfunction of the normal enzyme activity owing to genetic, hormonal or inhibitory-chemical influences. Secondly, are those forms arising from substrate overload of the enzyme activity (normal or depressed) such as an excess of dietary precursors of trimethylamine or variations in gut microorganisms resulting in enhanced liberation of trimethylamine substrate. Clearly, these are two intimately related aspects. A substrate burden that is easily handled by one individual may become a substrate overload in another that has a decreased enzyme function for whatever reasons (Mitchell, 1999). The following system of classification has been adopted in an attempt to allay this confusion (Mitchell and Smith, 2001).

Primary genetic form

This is probably the best understood of the various forms of the disorder and accounts for the majority of reported cases. It results from the failure of an isoform of the hepatic flavin monooxygenase enzyme (FMO3) to oxidize trimethylamine, provided via enterobacterial action on dietary precursors, into the non-odourous trimethylamine N-oxide. Recent work has shown that human FMO3 is highly polymorphic and some of the mutations, either alone or in combination, are associated with dysfunctional enzyme activity and the metabolic disorder, whereas other mutations appear to be benign (Dolphin *et al*, 1997; Treacy *et al*, 1998; Ackerman *et al*, 1999). In the near future it should be possible to employ gene array technologies to identify carriers (heterozygotes) for this condition.

Acquired form

There are at least three cases known of individuals with clinical and biochemical symptoms of fish odour where this condition appeared to emerge in adult life. In all instances, there was no previous history in childhood and there was no familial background to the disorder. What appeared common to the three subjects was evidence of hepatitis, possibly viral, in adult life and this may have been responsible for precipitation of the condition. However, once acquired, the condition did not appear to abate when the liver problems had resolved, suggesting some permanent damage perhaps at the genetic level (Ruocco *et al*, 1989; Mitchell and Smith, 2001).

Transient childhood forms

A report in the literature describes a premature infant (29-weeks old) developing a fish-like odour whilst being fed with a choline-containing food supplement. However, when the supplement was withdrawn the odour disappeared, and it failed to reappear at 8 months of age when the food supplement was reinstated. Similarly, when the supplements were given to three other preterm infants of similar age and weights, one of these developed a fish-like odour. The authors attributed the fish-like odour associated with choline-containing supplements to the immaturity of the N-oxidase enzyme (Blumenthal *et al*, 1980). Since then other cases of paediatric fish malodour syndrome have been described (Mayatepek and Kohlmuller, 1998; Zschocke *et al*, 1999).

Transient form associated with menstruation

Several female patients have reported anecdotally that their fish-like odour seemed to intensify with the onset of menstruation (Ayesh *et al*, 1993). A subsequent study of a single female fish malodour patient showed that her trimethylaminuric condition deepened just before the onset of menstruation and that this biochemical feature related closely to her own subjective descriptions (Zhang *et al*, 1996a). A systematic study has confirmed that in normal healthy women of menstruating age there occurs a short episode of trimethylaminuria just at the onset of and during menstruation that then disappears. By contrast, in male subjects there was no evidence of this cyclical variation (Mitchell and Smith, 2001).

Precursor overload

Trimethylamine is mostly derived from dietary presursors such as choline, carnitine and trimethylamine N-oxide through enterobacterial metabolism. Exposure to unusually high levels of such precursors may hasten a fish malodour syndrome especially if the individual is a haplotype for certain mutations. Large oral therapeutic doses of choline (8–20 g day^{-1}) have been used to treat Huntington's chorea, with patients complaining of a striking fish-like odour that was attributed to the generation of excessive amounts of trimethylamine

overloading their N-oxidation capacity (Growdan *et al*, 1977). Similar problems have also been reported following choline therapy for Alzheimer's disease (Etienne *et al*, 1978). It is noteworthy that precursors of trimethylamine, choline and lecithin (phosphatidylcholine), are sometimes recommended in quite high doses in health foods, food supplements and alternative diets.

Disease states

Impaired hepatocellular function or the existence of portosystemic shunts in patients with liver cirrhosis may interfere considerably with the first-pass metabolism and extraction of trimethylamine absorbed from the gut. The consequent increased blood levels of this amine observed in such patients may play a role in the development of hepatic encephalopathy and coma and associated *foetor hepaticus* (Wranne, 1956; Marks *et al*, 1978; Mitchell *et al*, 1999). In uraemic patients the abnormal overgrowth of bacteria in the small intestine greatly increases the liberation of trimethylamine from precursors within the diet. Compounded by reduced kidney function and subsequent decreased renal clearance, the amine levels in the blood increase, perhaps playing a role in nephritic neurological conditions as well as escaping via the breath (Simenhoff *et al*, 1977; Wills and Savory, 1981).

Concluding remarks

Since the presence of excess trimethylamine depends upon interplay of numerous events, the odour problems associated with this condition usually vary in intensity and may be transient in nature. When levels of trimethylamine are high, the volatile compound will leave the body via many routes (e.g. urine, sweat, breath, bodily secretions) bestowing upon the patient an odourous aura resembling that of rotten or decaying fish. However, when the levels of trimethylamine are lower, then the odour may only be noticeable arising from freshly voided tepid urine or exhaled on the warm breath. This may be particularly true for individuals who are heterozygous for the condition, in whom free trimethylamine only approaches threshold levels when several factors unfortunately occur in concert (Mitchell, 1999).

New cases of the 'fish odour syndrome' are being recognized constantly as an increased awareness of this metabolic problem permeates the medical and scientific communities. In terms of frequency of occurrence, it appears that the condition should no longer be regarded as 'rare' but more appropriately as 'uncommon' (Mitchell and Smith, 2001). The compromised ability to produce trimethylamine N-oxide has been detected in several ethnic groups and is not restricted to one area of the world's population (Table 2). A limited study in a British population has intimated that about one in 10 000 individuals may suffer from decreased N-oxidation capacity sufficiently severe to precipitate the clinical symptoms of the 'fish odour syndrome'. Although this particular study was small, applying simple Mendelian genetics and the Hardy–Weinberg quadratic suggests that up to 1% of the population may be heterozygous for this condition (Zhang *et al*, 1995). This is in broad agreement with indications from the population data (Table 2).

Table 2 Comparative incidence of dysfunctional N-oxidation of trimethylamine in various populations

		Subjects excreting less than 80% total material as N-oxide[a]			
Population	*Size*	*n*	*%*	*95% CI*[b]	*Reference*
British	421	6	1.4	0.3–2.6	Zhang *et al* (1996b)
Chinese	159	2	1.3	0.8–1.7	Lee *et al* (2000)
Ecuadorian	80	3	3.8	1.6–5.9	Mitchell *et al* (1997)
Jordanian	116	2	1.7	0.5–2.9	Mitchell *et al* (1997)
Jordanian	82	8	9.8	6.5–13.0	Hadidi *et al* (1995)
New Guinean	100	11	11.0	7.9–14.1	Mitchell *et al* (1997)
Thai	103	5	4.9	1.9–7.8	Thithapandha (1997)

[a]The amount of trimethylamine N-oxide is expressed as a percentage of the total 0–24 h urinary excretion of both trimethylamine and trimethylamine N-oxide. This is used as a measure of an individual's trimethylamine N-oxidation capacity.
[b]95% Confidence interval.

In instances where oral malodour, that may be transient in nature, cannot be assigned to problems within the oral cavity, where the levels of sulphur-containing volatiles in the exhaled breath are not diagnostically high and where no obvious extra-oral causation is present, mild cases of trimethylaminuria (fish odour syndrome), perhaps in the heterozygous state, should be considered as a possible causative factor.

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REVIEW

In vitro models for oral malodor

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A model is a representation of some real phenomena and contains aspects or elements of the real system to be modeled. The model reflects (or duplicates) the type of behavior (or mechanisms) seen in the real system. The main characteristic of any model is the mapping of elements or parameters found in the system being studied (e.g. tongue dorsum biofilm *in situ*) on to the model being devised (e.g. laboratory perfusion biofilm). Such parameters include correct physico-chemical (abiotic) conditions as well as biotic conditions that occur in both model and reality. The main purpose of a model is to provide information that better explains the processes observed or thought to occur in the real system. Such models can be abstract (mental, conceptual, theoretical, mathematical or computational) or 'physical', e.g. in the form of a real disaggregated *in vitro* system or laboratory model. A wide range of different model systems have been used in oral biofilm research. These will be briefly reviewed with special emphasis on those models that have contributed most to knowledge in breath odor research. The different model systems used in breath odor research are compared. Finally, the requirements for developing an overall 'bad breath model' from considering the processes as a whole (real oral cavity, substrates in saliva, biotransformation by tongue microflora, odor gases in the breath) and extending this to the detection of malodor by the human nose will be outlined and discussed.
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Keywords: experimental models; oral malodor; tongue biofilm; perfusion biofilm system

Introduction

What do we mean by the term 'model'?

A model is a representation of some real phenomena and contains aspects or elements of the real system to be modeled. The model reflects (or duplicates) the type of behavior (or mechanisms) seen in the real system. The main characteristic of any model is the mapping of the elements or parameters found in the system being studied (e.g. tongue dorsum biofilm *in situ*) on to the model being devised (e.g. laboratory perfusion biofilm). Such parameters include correct physico-chemical (abiotic) conditions as well as biotic conditions that occur in both model and reality.

Why do we need models?

The main purpose of a model is to provide information that better explains the processes observed or thought to occur in the real system. Their main value is therefore explanatory power or predictive capability. With regard to oral biofilms, their complexity is so large that it is difficult to study them without recourse to an experimental laboratory model. To study mechanisms or processes occurring in biofilms, it is necessary to perturb them. There is a limit to the amount of perturbation that can be done on a real tongue biofilm. Moreover, it is difficult to keep all other environmental factors controlled or constant. It is easier to carry out experiments in the confines of a well-equipped laboratory than to work in the human mouth. Moreover, in a laboratory system the environmental conditions can be controlled more precisely and the perturbation of the system can be controlled at will. This degree of control allows the operator to vary one particular parameter at a time and observe the resultant effects (called a 'unifactoral' approach). The response of the biofilm system can be determined across a wide range or set of environmental conditions and cause–effect relationships proposed, as clear answers to proposed questions or hypotheses. All the other components of the system are held constant or simply ignored. If the model turns out to be a good representation of, say, oral biofilm growth and metabolism it can then be used for other purposes such as screening compounds with interesting biological activity against biofilms (e.g. potential anti-plaque, anti-odor or anti-caries compounds) and predict their likely effects if given *in vivo*.

Types of model (physical and abstract)

Models can be abstract (mental, conceptual, theoretical, mathematical or computational) or 'physical', e.g. in the form of a real *in vitro* system or laboratory model. Even

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the oral cavity itself can be regarded as a model if perturbed and measured (or monitored) in a meaningful way.

Physical model

In terms of complexity, the physical (experimental) model may range from the most complex; *in situ/in vivo* (real oral cavity) to less complex (e.g. *in vitro* perfusion biofilm) to least complex (e.g. *in vitro* test tube reaction). Physical models may also be: static/dynamic, open or closed, steady-state or non-steady-state (see later examples).

Theoretical model (conceptual, mathematical and computer)

In practice, many practical or empirical models have theoretical counterparts that improve the explanation and predictive powers of the system. As an explanatory system a model is nearly always only partial (explaining just some of the processes or main steps) rather than encompassing the process *in toto*. All the conceptual ideas we have on biofilms are bound to be less than reality, so our conceptualizations can themselves be considered to be models in the abstract sense of the word (a so-called mental model). Even the theoretical models can be of different types. For example, a model may be deterministic (average behavior of the system) or stochastic (the collective and singular behaviors of the small elements or units – cells or molecules – that help make up the system using probability theory).

Black box vs white box

Most systems contain collections of interacting subsystems. With respect to the whole the parts are seen as subsystems. With respect to the parts, the whole is seen as a supersystem. It is possible to measure the overall behavior of the supersystem as a whole (by just considering its total input and total output), without being aware of all its parts or which part of the input goes to which subsystem. This approach considers the system as a 'black box', something that takes an input, and produces an output, without considering what happens in between. If in contrast all the system's internal processes are revealed, it can be called a 'white box'. Although the black box view may not be completely satisfying, in many cases this is the best we can get as many processes are still unknown and the only thing that can be clearly established is the final result. The 'black box' view is sometimes good enough but this may depend on the hypothesis set and the nature of the known/unknown processes. Most systems are gray boxes (elements of black and white). The black box view is not restricted to situations where the internal systems are unknown as even when known (or knowable) we may prefer to ignore the internal details. For example, when modeling volatile sulfur compound (VSC) production in biofilms, it may not matter which particular microbe produces VSC. It may be sufficient simply to know the total amount of substrate (cysteine) that enters the biofilm to estimate the total amount of VSC produced. The 'black box' view of the biofilm will be much simpler and easier to use for predictive calculation of overall VSC levels than the more detailed 'white box' view, which might trace the movement of every portion of substrate to every particular microbial cell in the system.

These two complementary views, 'black' and 'white', of the same system illustrate a general principle: systems are structured hierarchically. They consist of different levels. At the higher level, you get a more abstract, encompassing view of the whole, without attention to the details of the components or parts. At the lower level, you see a multitude of interacting parts but without understanding how they are organized to form a whole.

Computer-based or mathematical modeling

Using computers it is now relatively easy to summarize quantitatively certain outputs for a range of given inputs based around some mathematical equation. An example is the logistic equation for predicting biofilm growth:

$$\ln\left(\frac{k-x}{x}\right) = a - rt,$$

where x = biomass, a = constant, k = upper limit constant, r = growth rate constant and t = time.

However, this is a black box empirical approach with two or more unknown constants without any real world meaning. They do not relate to simple measures with real units of measurement. They are simply a series of mathematical descriptions of curves rather than a description of the real mechanisms. Although empirical systems can be made more complicated using more complex algorithms they bring little in the way of understanding and have limited use.

A true alternative is to build the model around more basic theoretical-mechanistic equations: e.g. the equations relating the relationships between reaction rate (or growth rate), substrate (or limiting nutrient) concentration and enzyme (or cell) affinity for substrate (Figure 2). This includes the Michaelis–Menton equation for enzyme reactions and Monod equation for whole cell transformations and growth. The clear advantage of the Michaelis–Menton or Monod model is that the parameters (V_{max}, V, s, K_m or μ_{max}, μ, s, K_s respectively) have real biological meaning and are experimentally accessible.

Any set of mathematical equations may constitute a mathematical model provided they aim at representing a real system and are based on some theory regarding the systems operation and are not just made to fit it in a purely empirical way.

Mathematical models that regard biofilms as homogeneous steady-state films containing a single species have been proposed (Rittmann and McCarty, 1980). This model has been evolved to cover dynamic multi-substrate-multispecies biofilm computer models (Wanner and Gujer, 1986; Rittmann and Manem, 1992; Wanner and Reichert, 1996). An approach using discrete cellular automata (to simulate the rules that govern the lives of microbial cells) has also been

employed to model biofilms (Wimpenny and Colasanti, 1997; Picioreanu *et al*, 1998). These allow the simulated biofilm structure to evolve as a self-organization process, emulating how real bacterial cells organize themselves into biofilms. These models produce realistic, structurally heterogeneous biofilms. Mathematical models of diffusion/reaction in dental plaque (incorporating Fick's law of diffusion) have also been described (Dibdin, 1981, 1997) as have models to predict the effects of antimicrobial activity within biofilms (Dibdin *et al*, 1996; Stewart, 1996).

Modeling terminology

In developing, constructing and using models to explain, predict and ask questions of real life processes, it is of fundamental importance that the purpose of, and assumptions underpinning, the model are clearly stated and understood. In addition, the assumptions made should be considered and their potential impact questioned whenever the model is used.

However, it is important that modeling strategies and associated procedures are clearly defined and adopted so that the quality and robustness of model output can be quantified and limits of applicability set. A vast body of literature, particularly for conceptual and computer-based models, has been written concerning the criteria that should be adopted in order to signify the usefulness of a given model. The concepts promulgated in such studies are transferable to use with 'physical models' and, as such, some especially important concepts are summarized in Table 1.

Table 1 Modeling terminology and procedures

Concept/term	*Description/process*
Reality	An entity, situation or system that has been selected for analysis (Schlesinger, 1979)
Conceptual model	Verbal description, equations, governing relationships or 'natural laws' that purport to describe reality (Schlesinger, 1979)
Verification	The process of ensuring that the implementation of the conceptual model is correct (Schlesinger, 1979) Substantiation that a model is in some sense a true representation of a conceptual model within certain specified limits or ranges of application and corresponding ranges of accuracy (Refsgaard and Henriksen, 2003)
Validation	The determination that the theories and assumptions underlying the conceptual model are correct and that the model representation of reality is reasonable for the intended use of the model (Sargent, 1988) Substantiation that a model, within its domain of applicability, possesses a satisfactory range of accuracy consistent with the intended application of the model (Refsgaard and Henriksen, 2003)
Turing test	The process whereby experts who are knowledgeable about the system in question (reality) are asked if they can discriminate between system and model outputs (Sargent, 1988)
Credibility	That there is a sufficient degree of belief in the validity of the model to justify its use for research and decision-making (Rykiel, 1996)

Considerations in modeling biofilms

1. Existing knowledge of the natural system being modeled: in our case we are interested in modeling tongue biofilm in terms of its growth and production of volatile compounds, particularly VSCs. The biofilm can be defined as a matrix-embedded microbial population where cells are adherent to each other and/or to other surfaces and interfaces. There are different forms of biofilm in respect of microbial composition and cell density distribution. For example, biofilms may range from patchy microcolonies of one or two species toward open spongy structures of microcolonies and communities with pores and channels, toward dense biofilms with highly packed bacteria. Unlike biofilms that form on hard surfaces, the tongue biofilm is thought to be relatively porous with respect of ingress of substrates and egress of products. Although there is a large amount of work published about VC/VSC production from various microbial species when fed different substrates there has been very little published about tongue biofilm structure and its properties (growth rate, biotransforming capacity) *in situ*. It is likely that in common with all biofilms, the tongue biofilm mode of existence will protect microbial cells against biocides and antibiotics and may be over 100 times more resistant than their planktonic counterparts.

2. Objectives: what it is that you want to find out about. Laboratory models enable the researcher to have control over: types of microbes, environment, nutrient supply and substrata. There are also increased options for experimental protocols, sampling and analysis. Different models are suitable for different purposes.

3. Biodiversity classes (Sissons *et al*, 1999) and source of microbes: class I: single species (monospecies) (e.g. *Staphylococcus epidermidis* on catheter); class II: 2–5, 6 or 7 (e.g. gram-negative species on urinary tract catheter); class III: 7–30 or so (eg. *Bioremediation consortia*); class IV: 30–500 or so (e.g. dental plaque); class V: 500–1000 or so (e.g. soil communities on clay particles). A microcosm is a laboratory subset of the natural population from which it originates. It retains the genetic, temporal and structural heterogeneity of the natural system and can evolve further in response to environmental changes.

4. Matching conditions in model and reality (mapping of biotic and abiotic conditions). In order for an experimental model to closely resemble and behave like the real biofilm *in situ* there is an obvious need to map the chemico-physical environment (temperature, pH, nutrients and inhibitors). The abiotic (chemico-physical) parameters include: temperature (35–37°C), pH (6.5–7.5), osmolarity (equal to that of saliva), type and concentrations of nutrients or substrates and type and concentrations of inhibitory compounds. For experimental purposes we can use a spectrum of nutrient molecules from one main carbon/energy (C/E) substrate (e.g. glucose) to a complex mix containing sugars, polysaccharides, amino acids, peptides, and proteins. Moreover, the medium could be synthetic-defined or complex (e.g. artificial saliva *vs* real saliva filtrate).

The biotic parameters include the diversity of living entities and again this can be a spectrum from monoculture to real inoculum (complex microcosm).

5. Growth rates and cell densities similar to those *in situ* (oral cavity).

6. Fluid regime (pulse or dose, defined or undefined media) and hydrodynamics (flow and shear: turbulent, plug and laminar). The fluid regime will also include treatment protocols (antimicrobials, inhibitors of VSC production).

7. Substrata and conditioning film: important if studying initial formation of biofilms. Hydroxyapatite and salivary pellicle might be considered for plaque biofilm whilst salivary-coated polymeric material could be used to simulate the tongue surface.

8. Sampling protocol: e.g. number of replicate samples; pre-, postperturbation or continuous monitoring?

9. Analytical techniques (measurements of pH, reducing power, metabolic end-products, substrate utilization and enzyme assays). Discontinuous samples or continuous (real time) monitoring.

Models currently used in oral biofilm research

There are a range of laboratory experimental physical models used to study oral microbes and biofilms, from simple batch culture closed systems (using test tubes, closed flasks or wells in microtiter plates) to open, continuous flow arrangements. *In vivo* or *in situ* experiments (e.g. studying VSC production following cysteine rinses) may relate well to the natural situation and reveal important information. However, only limited measurements and perturbations are possible in a real human mouth. Computer-based modeling using inputs based on real empirical data can summarize quantitatively what is known about biofilm properties and may give useful insights to help generate and test hypotheses. For studying biofilms in general and oral malodor in particular, a number of different models or systems have been used *in vitro*; some of these will be briefly described.

Closed systems

1. Incubated saliva or salivary sediment or incubated species of selected microbes as cultures. These experiments tell us something about the potential of species and/or microcosms to do particular types of transformation. It is clear from previous work using closed systems (Tonzetich, 1971; McNamara *et al*, 1972; Tonzetich and McBride, 1981; Kleinberg and Codipilly, 1995) that anaerobes, and particularly the gram-negative anaerobes (GNA), tend to produce a higher degree of malodorous VC/VSC in culture than other groups. By incubating species of oral microbes in liquid culture containing elevated levels of amino acid substrates, Kleinberg and Codipilly (1995) were able to demonstrate odor production by a range of species against a range of substrates. In general, gram-positive species were not particularly effective at producing odor whilst gram-negative species, particularly the anaerobic species *Porphyromonas gingivalis* and *P. intermedia*, were effective odor producers. The most effective substrates inducing odor were cystine/cysteine, methionine, ornithine, lysine and tryptophan. *Porphyromonas gingivalis* produced increased odor with all these substrates which implies that it may have the potential to produce hydrogen sulfide, methyl mercaptan, putrescine, cadaverine and indole from respective substrates. A more objective analysis of VC and VSC production by oral species has been made using gas chromatography mass spectrometry measurements of culture headspace (Kostelc *et al*, 1980; Greenman, 1999) and data show that a wide variety of volatile chemical species are produced from a wide range of species/samples.

Batch cultures are simple and may be adequate for gaining certain types or levels of knowledge but they have major limitations. In batch culture, the whole physico-chemical environment (number of cells, concentrations of substrates, nutrients, products, pH and oxygen tension) is constantly shifting as an inevitable consequence of growth in a closed system. The conflated variables disallow strict cause–effect relationships to be discerned between set environmental conditions and measured physiological responses.

2. Cell suspension assay (non-growth system; initial rates measured using defined assay). Provided these assays are of short duration they can give quantitative rate data (K_m, V_{max}) using fresh samples (e.g. tongue scrape material). In contrast to the previous system they are suspension assays not involving growth/culture and are generally more useful than the previous model.

Open systems (continuous flow devices)

Table 2 describes a number of different systems that have been used in the past and the reader is referred to original papers (Table 2) to find out more details.

Chemostat

The chemostat is an ideal device for generating steady-state homogenous cultures and has been used widely in oral microbiology research (Marsh *et al*, 1983; McKee *et al*, 1985; McDermid *et al*, 1986), including research on oral malodor (Greenman, 1999). However, its use for studying biofilms is very limited as it operates on the principle that all cells remain in suspension (planktonic growth) and if biofilms do form within the system, they disturb the steady-state. Nevertheless, chemostats are often used to provide steady-state cell suspensions to subsequently feed into a flow device (Herles *et al*, 1994; Li and Bowden, 1994). A two-stage chemostat system with test surfaces immersed into the second-stage vessel has been applied to the study of biofilm formation (Keevil *et al*, 1987; Keevil, 1989; Marsh, 1995).

Flow cell systems

Continuous flow cell (slide) models have been used widely (Table 2) to study biofilm structure, especially initial events of formation. They often employ microscopy such as confocal laser scanning microscopy to capture and analyze images over time. Although useful for studying attachment and early stages of growth and ecology they are not particularly suitable for studying

Table 2 A selection of *in vitro* biofilm systems (some applied to oral biofilms; a few applied to malodor research)

Biofilm system	*Applications*	*Authors*
Artificial mouth models using enamel or hydroxyapatite surfaces	Colonization, biofilm metabolism, effects of antimicrobial compounds	Russell and Coulter, 1975; Coulter and Russell, 1976; Dibdin *et al*, 1976; Sissons and Cutress, 1987; Zampatti *et al*, 1994; Sissons, 1997; Soukos *et al*, 2000
Calgary biofilm device	Antimicrobial susceptibility	Ceri *et al*, 1999; Spoering and Lewis, 2001
Robbins device	Antimicrobial and biocide efficacy	McCoy *et al*, 1981; Gristina *et al*, 1987; Hoyle and Costerton, 1991
Modified Robbins device	Antimicrobial susceptibility	Khardori *et al*, 1991; Larsen and Fiehn, 1995; Yassien *et al*, 1995
Continuous culture flow cell chambers	Mixed biofilm cultures and metabolic interactions; formation, ecology, cell death; efflux pumps; antimicrobial susceptibility;	Keevil *et al*, 1987; Herles *et al*, 1994; Li and Bowden, 1994; Wolfaardt *et al*, 1994; Marsh, 1995; Møller *et al*, 1998; Heydorn *et al*, 2000; De Kievit *et al*, 2001; Neu *et al*, 2002
Fluidized bed environments and airlift reactors	Biofilm structure; biotransformations	Gjaltema *et al*, 1995; Tijhuis *et al*, 1996
Perfused biofilm fermenter	Antimicrobial susceptibility; cell surface hydrophobicity; extracellular polymeric substances (EPS) production	Gilbert *et al*, 1989; Allison *et al*, 1990a,b, 1999; Duguid *et al*, 1992a,b; Evans *et al*, 1994
Constant depth film fermenter	Biofilm formation, ecology; effects of antimicrobials (including effects on oral malodor)	Kinniment *et al*, 1996; Wilson, 1996; McBain *et al*, 2003a,b,c; Pratten *et al*, 2003
Sorbarod system	Characterization of pure and mixed species biofilms; antimicrobial susceptibility; effects of cysteine on VSC (oral malodor)	Hodgson *et al*, 1995; Budhani and Struthers, 1997; Foley and Gilbert, 1997; Gander and Gilbert, 1997; Gander and Finch, 2000; Maira-Litrán *et al*, 2000; Greenman *et al*, 2002; Nelson *et al*, 2003; Spencer *et al*, 2003
Immersion of surfaces in chemostat cultures	Antimicrobial susceptibility	Anwar *et al*, 1989; Keevil, 1989
Rotating annular reactors, rototorque and biodrum	Colonization, ecology (shear stress)	Gjaltema *et al*, 1994; Arcangeli and Arvin, 1995; Allison *et al*, 1999; Lawrence *et al*, 2000

VC/VSC biotransformations so are generally not used in malodor research.

Larger-scale models

The constant-depth film fermenter (CDFF) has been widely used in studying biofilms (Table 2). In essence, this system allows biofilms to form in small recesses which are periodically swept with a blade to remove any material above the level of the recess. In this way it keeps the biofilm at a constant depth. All other physico-chemical parameters (medium, flow rate, atmosphere, temperature and pH) are carefully controlled. Although this system has many advantages over flow cells (especially for studying the properties of mature biofilms and the effects of substrates and antimicrobials) it has one major disadvantage; the growth rate of cells is ill-defined. Two parameters contribute to the growth of the biofilms; growth from within and further attachment of cells from without. This model is not able to separate the contributions made from these two parameters. Nevertheless, CDFF have been used to study the effects of inhibitors on VSC production by mixed culture microcosms of oral origin (Pratten *et al*, 2003).

Perfusion systems

Membrane bioreactors have been described elsewhere (Helmstetter and Cummings, 1963). The perfused biofilm fermenter (Gilbert *et al*, 1989) incorporates a bacterial cell impermeable membrane (e.g. Swinnex filters produced by Millipore Corporation) which is fed from one side with sterile growth medium. The microbial cells are attached to the other side of the filter (the underside). The attached microbes grow at a rate depending on the composition and flow rate of the medium. As cells grow, the offspring are released into the waste medium (called permeate, eluate or perfusate). By measuring the rate of cells released from the system and knowing the attached biofilm cell population the growth rate can be calculated. The growth rate can be controlled by changing the flow rate of medium. However, the membrane system is small and the corresponding samples that can be obtained for analysis are also small in volume and numbers of cells.

A more recent development is a modified version of the perfused biofilm fermenter termed the Sorbarod system (Hodgson *et al*, 1995). This has a biofilm surface area which is many times greater than in the membrane method and produces sufficient sample to allow biochemical determinations to be more conveniently made.

One important advantage of the Sorbarod perfusion model is that it is an open system which for a significant period of time (*c.* 3–4-day period) can be considered to be in steady-state when presented with a favorable physico-chemical environment (flow rate, media composition, temperature and pH). A second advantage is that the physical model itself is of a size that allows adequate samples to be taken for analysis without the sampling procedure disturbing the steady-state. With electrodes present it can be constantly monitored (Figure 1). Another important advantage with the *in vitro* perfusion model is that it has a

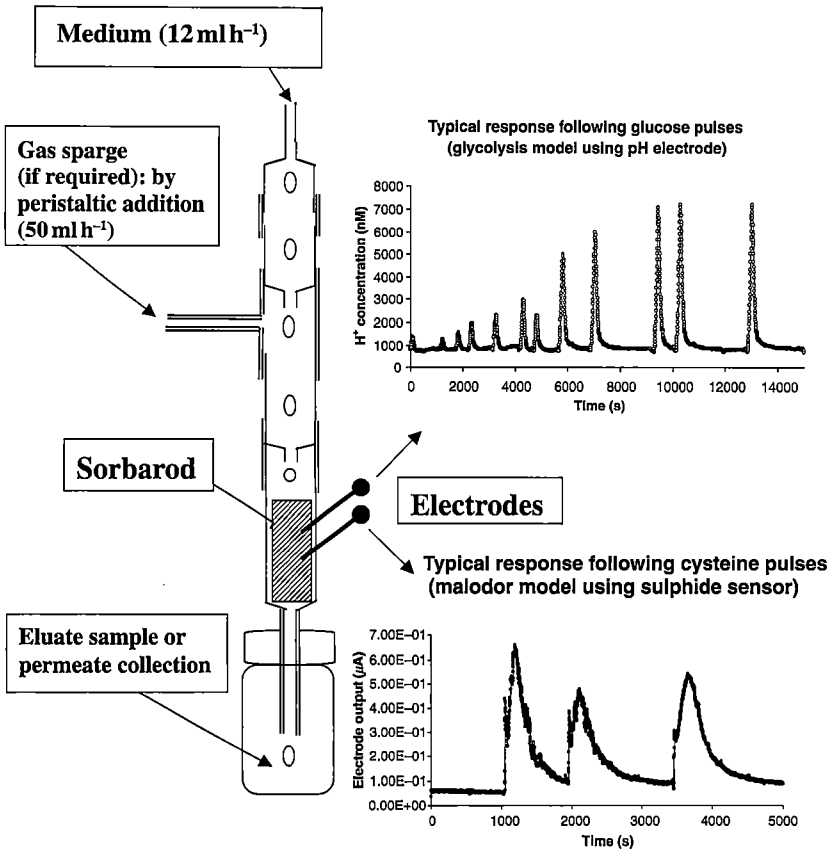
Medium (12 ml h⁻¹)
Gas sparge (if required): by peristaltic addition (50 ml h⁻¹)
Sorbarod
Electrodes
Eluate sample or permeate collection
Typical response following glucose pulses (glycolysis model using pH electrode)
H⁺ concentration (nM)
Time (s)
Typical response following cysteine pulses (malodor model using sulphide sensor)
Electrode output (μA)
Time (s)

Figure 1 Sorbarod biofilm system

theoretical or mathematical counterpart giving a computer model of the same processes (Figures 2 and 3). The model can also be used for screening new types of antimicrobial compounds or anti-breath agents and comparing their efficacy against established agents against biofilm growth and VC/VSC production in the same model.

A perfusion biofilm incorporating a Sorbarod was employed to study complex mixed culture biofilms derived from tongue scrape inoculum (Greenman *et al*, 2002; Spencer *et al*, 2003). These biofilms were examined in terms of their ecological stability by sampling eluates and biofilms and recovering component species using conventional microbiological techniques (selective and non-selective recovery medium). The physiology of VSC production by the biofilm cells was studied using a cell suspension assay coupled to a halimeter. A quasi steady-state ($\mu = 0.014\ h^{-1}$) was achieved by 48 h and continued to the end of the experimental period (96 h). Comparison of biofilms with eluate and original inoculum showed their ecological profiles to be similar showing that the model maintained good ecological stability. Addition of S-substrates (cysteine, glutathione and methionine) to the growth medium resulted in samples with higher specific activity toward respective VSC substrates than controls thus demonstrating up-regulation (induction or de-repression) of VSC producing enzymes. Eluate and biofilm-derived samples were equivalent with regard to VSC-specific activities.

Some ideas for developing an overall 'bad breath model': 'what goes in must come out'

The most natural model is the natural ecosystem itself; the tongue biofilm *in situ*. The question remains, is it possible to model the overall process of oral malodor? The oral cavity is characterized by having three physical interactive phases: gas, liquid and solid. The gas/liquid mobile phases are highly dynamic and rapidly replaced. The replacement is described by the dilution rate, *D*, defined as the flow rate of gas or saliva divided by the volume of the oral cavity (for gas) or the residual salivary volume (for liquid).

Gas

The gas phase is characterized by rapid replacement and mixing. The dilution rate is about 40–80 h^{-1} although this is obviously reduced by breathing through the nose and shutting the mouth; it is increased by talking or (with blocked nose) breathing through the mouth.

Liquid

Mainly saliva, but some crevicular fluid (small volume but high concentration of S-containing substrates). The bulk fluid flows through the oral cavity at a dilution rate between 1.0 and 10.0 h^{-1}.

Solid

The solid phase consists of the tongue biofilm itself and the epithelial surfaces (which slowly exfoliate). The

A. Growth

Monod equations:

1. $\mu = \mu_{max} \frac{S}{K_s + S}$

where μ = specific growth rate,
μ_{max} = maximum specific growth rate
S = concentration of growth limiting substrate
K_s = growth substrate saturation constant

2. $Y = \frac{dX}{dS}$

where Y = growth yield
X = biomass concentration

Rate of growth in sorbarod and in tongue biofilm:

μ = rate of elution of cells (flow rate × cfu ml^{-1} h^{-1}) / biofilm population (cfu)

B. Enzyme reaction (biotransformation)

Michaelis–Menten equations:

1. $V = V_{max} \frac{S}{K_m + S}$

where V = initial rate of reaction
V_{max} = maximal enzyme velocity
S = concentration of rate limiting substrate
K_m = enzyme substrate saturation constant (Michaelis–Menten constant)

2. For an enzyme-immobilized bioreactor with well-mixed continuous flow:
Substrate flow in = substrate flow out + substrate consumption by reaction

$$QS_o = QS + k_r ES / K_m + S$$

where Q = volumetric flow rate of bulk fluid
S_0 = initial substrate concentration
S = final substrate concentration
k_r = rate of reaction (where $V_{max} = k_r E$)
K_m = Michaelis–Menten constant
E = enzyme concentration

C. Inhibition of enzyme (e.g. by anti-odor mouthwash; non-competitive inhibition)

$V = V_{max} \frac{S}{\alpha(S + K_m)}$

where $\alpha = 1 + i/K_i$
K_i = enzyme inhibition constant
i = inhibitor concentration

D. Inhibition of growth of cells in biofilm (e.g. by antimicrobial agent)

$\mu = \mu_{max} \frac{S}{\alpha(S + K_s)}$

where $\alpha = 1 + i/K_i$
K_i = growth inhibition constant
i = inhibitor concentration

Figure 2 Some equations of use in modeling *in vitro* or *in situ* biofilm growth and oral malodor

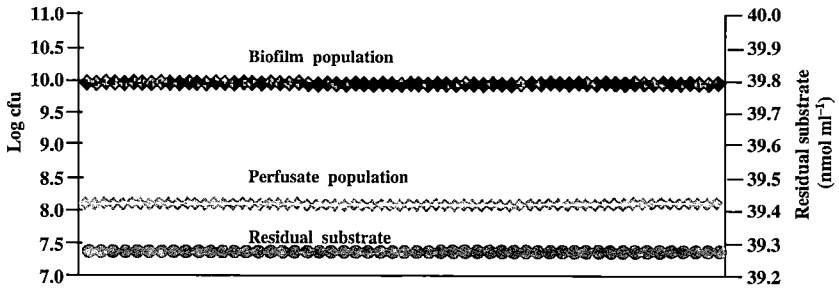

Figure 3 Computer (spreadsheet) model of the Sorbarod biofilm growth. Modeling was based on Monod's equation relating growth and substrate concentration. The perfusate flow rate chosen (24 ml h^{-1}) resulted in a growth rate of 0.018 h^{-1}

tongue architecture is complex with crypts and fissures supporting a high bacterial population. The biofilm is responsible for the biotransformations of immediate substrates to VC/VSC product and may also be responsible for increasing the amount of immediate substrates by enzymatic hydrolysis of protein or peptide secondary substrates.

In vitro studies suggest that the most important groups of organisms generating VCs and VSCs are anaerobes, particularly those GNA isolated from the tongue biofilm. Studies on human volunteers have revealed a correlation between subjects' oral malodor scores and log numbers of anaerobes, GNA and sulfide-producing organisms per unit area of tongue

surface (Hartley *et al*, 1996a). This relationship is also observed following the use of a metronidazole mouthrinse to selectively target the anaerobes (Hartley *et al*, 1999), where odor reduction and concomitant reduction of anaerobes and sulfide-producing organisms is highly significant ($P < 0.01$ at 24 h post-rinse). The mean number of bacteria on the tongue surface has been determined to be approximately 2×10^9 cm^{-2} (Hartley *et al*, 1996b). The macro-surface area of the tongue dorsum is approximately 30 cm^2. The resident tongue population is therefore approximately 6×10^{10} cells.

For studying cell growth we can consider the oral cavity as a continuous perfusion biofilm system. Saliva brings in nutrient substrates which allow the microbes to grow. Saliva also removes a portion of all components (eroded cells, low and high-molecular-weight products and residual substrates). As a first approximation it is reasonable to assume that the microbes grow in steady-state where their growth rate equals their removal rate. The removal rate is equal to the salivary total bacterial count per milliliter multiplied by the flow rate in milliliter per hour. According to Hartley *et al* (1999) the salivary bacterial count is approximately 2×10^8 per milliliter. Based on an unstimulated salivary flow rate of 12 ml h^{-1}, the total number of bacteria shed per hour equals 2.4×10^9. The production rate (growth rate, μ) is therefore: $2.4 \times 10^9 / 6.0 \times 10^{10} = 0.04$ h^{-1}.

Besides the addition of nutrients for bacterial growth and removal of bacterial cells shed from the surfaces, saliva also brings in sulfur-containing substrates (e.g. cysteine) which are biotransformed into VSC gases (e.g. H_2S). In steady-state, the production rate is equal to the removal rate (gas dilution rate). For studying VSC production, we can consider the oral cavity to be a continuous immobilized enzyme system (Figure 2). The amount of enzyme and its turnover is proportional to the amount of bacterial cells and their turnover and will be roughly constant.

The above considerations and equations shown in Figure 2 are the type that may be used to model bad breath by computer. To test out any computer model that may be developed, real data from real experiments in the human mouth will be needed. For example, cysteine rinses may be given to human volunteers and the kinetics of VSC production monitored using breath sensors.

Malodor on the breath is ultimately perceived by another person via the nose of the perceiver. This process can also be modeled. Although a kinetic model has yet to be proposed, an equilibrium model has now been published (Greenman *et al*, 2004).

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ORIGINAL ARTICLE

Malodour in denture wearers: an ill-defined problem

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Denture plaque has not been studied to the same extent as dental plaque, and although there are many similarities in microbial composition, there are some significant differences. Denture-induced stomatitis is associated with poor denture hygiene, a more acidogenic plaque and the presence of *Candida albicans*. Obligate Gram-negative anaerobic micro-organisms, although present in denture plaque, have rarely been specifically investigated. Opportunist pathogens including coliforms and staphylococci have been isolated from dentures. Teeth adjacent to partial dentures are more susceptible to caries and periodontal diseases, perhaps due to an increased plaque buildup at the prosthesis/tooth interface. Little work has been published on malodour associated with dentures. The inert material provides a substratum for the plaque biofilm, which encompasses a range of odour-producing species. The microbiology of the tongue in denture wearers has not been specifically studied. Thus the nature, origin and extent of malodour in denture wearers is ill-defined, but many species capable of producing malodorous compounds are present. The wide age and health range presented by denture wearers further confounds investigation. There is a need for further work in the area, both for cosmetic- and health-associated reasons in the increasing elderly population.
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Keywords: dentures; malodour; denture plaque

Introduction

A significant proportion of the adult population wears complete or partial dentures. The factors associated with tooth loss – dental caries, loss of periodontal support, a history of dentoalveolar trauma, a history of dental care – are additive over time, thus denture wearing is more associated with an older population. In the past, false teeth were sourced from ivory, or animals, or the poor – whose diet was less damaging to the dentition than that of the wealthier population. These prostheses of biological origin were subject to caries and spoilage: the fan thus became a useful aid to hide the sight and smell of both false and true dentition! The origin of denture odour is now potentially less diverse and more easily controlled. Dentures are now made of synthetic polymers, particularly polymethacrylate, using casts constructed from impressions taken in the mouth. Soft liners, or more temporary tissue conditioners, are applied to the prosthesis to improve comfort and fit. Denture adhesives are also commercially available.

Thus a range of inert materials placed in close proximity to oral tissues over extended time periods provides a hard, non-shedding substratum in a relatively stagnant environment, enabling the attachment and colonization of micro-organism and the formation of denture plaque. The oral condition particularly associated with the wearing of dentures is denture-induced stomatitis. The bulk of the literature concerning denture plaque focuses on the aetiological agent, generally deemed to be *Candida albicans*, and associated causative factors, particularly poor denture hygiene and consequent plaque (and calculus) accumulation (Verran, 1999). Malodour associated with dentures is rarely addressed directly in the literature, although reference is frequently made in passing (Myatt *et al*, 2002), and many denture-cleaning products claim breath freshening properties. The implication behind these claims is that there is a potential odour problem, yet no evidence could be found in the literature of a comprehensive study on the topic. This paper brings together some of the evidence associating denture wearing with possible malodour, and outlines some of the areas where further work is needed.

Demographics

Numbers of denture wearers are significant, and it is generally recognized that the elderly population is increasing in many of the developed countries. Currently there are 60 million denture wearers in Europe, 15 million of them in the UK. Of these, approximately 40% wear full dentures, 20% one full denture only, 17% one full denture and one partial, and 23% partial dentures only. In the US, in 1900, 3% of the population were over 65: in the year 2000, the figure had risen to over 13% (corresponding to 37 million individuals, of whom

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4 million were over 85). In Japan, it is predicted that the elderly will comprise over 25% of the total population by 2025 (Shay, 2000; Senpuku *et al*, 2003). India has a large geriatric population (60+) of 77 million, comprising almost 8% of the population (Shah *et al*, 2004).

Clearly there are many issues to be addressed as a result of these significant demographic shifts, and perhaps denture malodour is not amongst those of most concern. However, oral health is an important aspect to the care of the elderly, not only those who are hospitalized (O'Reilly, 2002), but also those in residential homes where socializing and eating tend to occur together (Shay, 2000), or in their own homes, where odour, either perceived or actual, presents embarrassment and discomfort to the individual and to their family and friends.

Preservation of some or much of the dentition into old age has become more common in recent decades. However, the growth of the proportion of the population living in their 70s, 80s, 90s and beyond results in a continued growth in the number of older persons requiring replacement dentition, even as the proportion of older people requiring dentures has declined (Gordon, 1989). Thus there will be a denture-wearing population for some time to come.

The denture-wearing population could be divided into two groups: the active and debilitated elderly. The former enjoy a full and active social life, and present a significant consumer base. Denture and oral hygiene products would be in demand for this group, primarily for aesthetic purposes. The debilitated and more aged elderly are unable to carry out their own oral care procedures, and require assistance to do so in order to maintain the best possible oral health status. A knowledge of the significance of malodour in the denture-wearing population, of factors involved in the cause, and of any significant differences between malodour in denture wearers and the dentate population, would enable the development of appropriate treatment, control (and marketing) strategies to address the needs of these two very different denture-wearing groups.

Oral microbiology in denture wearers

The oral flora of the elderly has been likened to that of the baby (Socransky and Manganiello, 1971), the main similarity being the lack of natural teeth. However, in terms of malodour, differences would be anticipated, for example due to the effect of the lifelong diet and health experience of the adult (Percival *et al*, 1991). If the origin of malodour is the tongue flora, then one might anticipate some alterations in the tongue flora with age: the tongue is deemed to be a reservoir for many oral species, being present in the mouth before and after teeth have erupted and departed!

In comparison with the dentate individual, the mouth of the denture wearer presents additional sites and environments to support the growth of micro-organisms. For example, teeth adjacent to partial prostheses are more prone to caries and gum disease, presumably due to the enhanced accumulation of plaque held close to the host tissues by the inert denture (Verran, 1999).

The tongue

The tongue supports a significant microbial population (Hartley *et al*, 1999), but the nature of the tongue biofilm and coating in denture wearers has not been studied, thus comparisons with the composition of the tongue in the dentate cannot be made. One might speculate that there would be an increased likelihood of isolating *C. albicans*, as there is a significant carriage of the yeast on dentures. As full denture wearers have no gingival crevices, and no periodontal disease, one might presume the absence of periodontal species: would they then be present on the tongue? Certainly *Fusobacteria* and *Prevotella* species have been isolated from denture plaque (Gusberti *et al*, 1985). In our laboratories they have been particularly associated with more mature plaque (Figure 1). The involvement of these micro-organisms (and others) in classic oral malodour surely merits some investigation of their presence in the relatively unexplored milieu of denture plaque. It should also be noted that the composition of a 'healthy' tongue biofilm in the dentate has yet to be defined in any detail.

Oral epithelia

Lip and cheek cells are in transient contact with denture material rather than teeth. Cells will be shed from these surfaces, and biofilm will not accumulate. However, some of the oral epithelia will be occluded by the denture-fitting surface. Inflammation of the maxillary mucosa in denture-induced stomatitis results from the activities of micro-organisms in the adjacent denture plaque.

Denture

The prosthesis itself will support the development of denture plaque and calculus, but presents different environments to the colonizing species. The fitting surfaces are exposed to high nutrient concentrations and low salivary flow rates, and provide a roughened topography to support and protect the plaque (Figure 2). *Candida albicans* is found more frequently on this fitting surface (Verran, 1988). The non-fitting surface is more exposed: plaque tends to accumulate at the 'tooth–gum interface' (Figure 3) much as it does on natural teeth, again due to the protection from shear provided by the surface features.

Denture soft lining materials

Denture soft lining materials present a rubbery and resilient surface to the colonizing micro-organisms. These materials may also leach plasticizer and harden over time. The uneven and potentially porous surface provides an excellent environment for colonization by micro-organisms. Evidence of penetration by yeast has been demonstrated *in vitro* over a 6-week incubation period, but over a 3-month in-use period this phenomenon was not evident, presumably due to the competition provided by other oral species, particularly

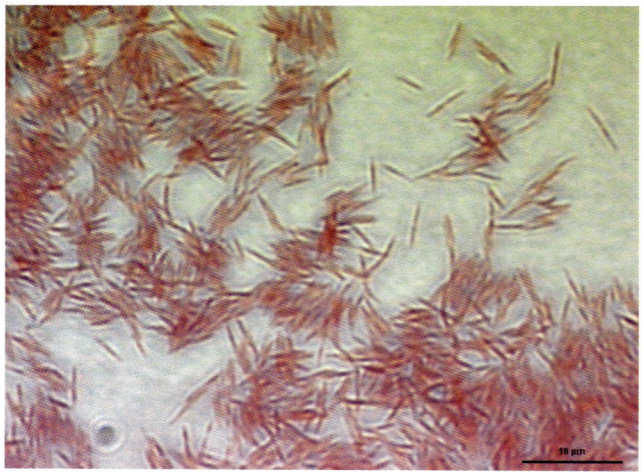

Figure 1 Gram stain of *Fusobacterium nucleatum* isolated from denture plaque, identified by 16sRNA

Figure 2 Disclosed plaque on a denture. Accumulation at the 'tooth–gum interface' is apparent

Figure 3 Undisclosed plaque on the fitting surface of a lower denture

bacteria, absent from the *in vitro* study (Bulad *et al*, 2004). Nevertheless, the fillers are potential substrates for the growth of some micro-organisms, and the accumulation of micro-organisms within the materials will provide a further opportunity for the deterioration and the generation of odour.

Denture plaque

There have been relatively few studies on denture plaque microbiology, the bulk being carried out in the 1980s. Images of denture plaque from the upper fitting surface reveal the presence of a pellicle, a typical biofilm morphology of columnar microcolonies, surmounted by occasional epithelial cells from the maxilla (Budtz-Jorgensen *et al*, 1980).

The composition has been deemed similar to dental plaque particularly that on the occlusal surfaces (Theilade *et al*, 1983; Theilade and Budtz-Jorgensen, 1988). Gram-positive cocci and rods dominate, with Gram-negative rods and yeast less common. Few obligate anaerobes are described (Gusberti *et al*, 1985). These studies comprise a very significant amount of microbiology, but inevitably include only a few individuals, and different sites in the mouth (Budtz-Jorgensen and Theilade, 1983): large variations in the populations of both were observed. Recent studies are few (Verran, 1999). In published work, the isolates obtained are often a direct consequence of the isolation media used. Many publications focus on *Candida* (Kulak *et al*, 1997; Nikawa *et al*, 1998) which comprises only 0–0.45% of the population numerically (Theilade *et al*, 1983) (although its cell mass is more significant). Thus other groups of organisms may be overlooked. This is particularly true for the obligate anaerobes, which are important if oral malodour is the focus of a study (Nakano *et al*, 2002; Tyrrell *et al*, 2003).

Some micro-organisms which are unusual in the oral flora but have been isolated from dentures include respiratory pathogens such as *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp. (Sumi *et al*, 2002; Senpuku *et al*, 2003) and staphylococci (including MRSA, Rossi *et al*, 1995). In one study, 48% of dentures sampled harboured members of the Enterobacteriaceae (Goldberg *et al*, 1997; Senpuku *et al*, 2003). Inhalation pneumonia is a common cause of death amongst the elderly debilitated, thus the role of the denture in harbouring such potential pathogens may be significant. Their transient nature (Eliason *et al*, 1992), or otherwise, is unknown.

There is a need for a larger and more comprehensive study on the oral flora of the elderly. Current knowledge in the field often relies on older publications, using relatively few subjects, sampling few sites and targeting specific micro-organisms. More modern molecular methods such as density gradient gel electrophoresis might well demonstrate differences in the total flora, cultivable or otherwise, in different sites in the mouth, in test populations.

Malodour

No evidence could be found in the literature of a study on denture wearers' perceptions of odour associated with denture wearing. One would assume that the adherent plaque on dentures would be unaesthetic in

terms of appearance, tactile sensation, taste and odour, but impairment of taste and smell perception under circumstances of poor denture hygiene has been noted (Hyde *et al*, 1981) implying that individuals may become inured to ongoing unpleasant taste and odour. Dentists and dental technicians comment in passing on odour (personal observations), but the bouquet has not been described or characterized. One reference to a 'sweet but offensive' odour was found (Goldberg *et al*, 1997).

The mouth and the denture may have different odours, but this has not been investigated. Salivary flow will be reduced beneath the denture: this property has been shown to increase the presence of volatile sulphur compounds (VSCs) (Koshimune *et al*, 2003). The source of odour is potentially diverse, as a wide range of odour-producing organisms have been isolated, including members of the Enterobacteriaceae, pseudomonads, Gram-negative anaerobes and yeast, but has not been explored to any great extent (Goldberg *et al*, 1997). The nature of the odour has not been defined. Studies on the breath-freshening effect of denture fixatives could not demonstrate a beneficial effect of the product on VSC production as determined by the halimeter, but a subjective organoleptic assessment proved more useful (Myatt *et al*, 2002). This indicates that malodour associated with dentures is potentially more complex (Table 1), or at least different from, and less well defined than, that in dentate individuals.

Control of malodour associated with dentures

Denture hygiene is the obvious method for ensuring that the denture remains clean. The British Dental Association recommends that to 'keep gums and teeth healthy to avoid bad breath' the denture (and the mouth) is cleaned every day. After being removed from the mouth, the denture should be cleaned over a basin of water (to prevent accidental breakage on dropping). It is recommended that the denture is left out of the mouth for at least 4–6 h, preferably 8 h, in every 24 h (http://www.bda.org). However, it should be recognized that many denture wearers, for reasons of vanity, do not leave their dentures out at night. There are several oral hygiene products available for use by denture wearers. Mouthwashes, chewing gums, toothpastes and denture fixatives all claim to freshen the mouth and keep the prosthesis clean (Shay, 2000; Myatt *et al*, 2002; Simons *et al*, 2002). Regular dental checks are also recommended to ensure that the tissues remain healthy, and that the denture fit is appropriate. However, as malodour associated with dentures is not a well-defined or recognized condition, nor is it perceived as a significant problem, specific approaches for its control or prevention are sidelined by work in more potentially health threatening, or commercially lucrative, areas.

In conclusion, studies on microbiology, and determination of the origin and nature of any malodour associated with denture wearing remains a neglected area in the comprehensive literature on oral microbiology, and the increasing literature on oral malodour, and merits further study (Table 2) in order to benefit the diverse relevant population.

Table 1 Factors associated with malodour in denture wearers (excluding odour of extra-oral origin)

Habitats
The denture and its plaque: the denture has a definite and distinct odour on removal from the patient's mouth
The tongue: the tongue/tongue coating/postnasal drip is generally deemed to be the origin of oral malodour
Substrates
Saliva
Food particles trapped beneath denture
Components of biomaterials
Micro-organisms
Gram-negative volatile sulphur compound (VSC) producing anaerobes on the tongue and in denture plaque
Gram-negative respiratory pathogens (*Klebsiella* and *Enterobacter* spp. produce VSC and cadaverine)
Yeast

Table 2 Factors to be considered in clinical studies on malodour associated with dentures

Differentiate between dentate, partial dentate and edentate population
Determine denture wearing and hygiene habits
General health status, nutrition, age, smoking, alcohol, medication and mobility
Denture status – cleanliness, material and soft liner
Whole mouth odour compared with denture odour
Denture microbiology compared with tongue and saliva microbiology (sampling)
Define nature of odour (gas chromatography-mass spectrometry, VSC and sensors)
Organoleptic training for denture-associated odour detection
Microbial origin of odour

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MINI-REVIEW

The rationale and potential for the reduction of oral malodour using *Streptococcus salivarius* probiotics

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The primary treatment for oral malodour is the reduction of bacterial populations, especially those present on the tongue, by use of a variety of antimicrobial agents or mechanical devices. However, shortly after treatment the problematic bacteria quickly repopulate the tongue and the malodour returns. In our studies, we have used a broadly-active antimicrobial (chlorhexidine) to effect temporary depletion of the oral microbiota and then have attempted to repopulate the tongue surface with *Streptococcus salivarius* K12, a benign commensal probiotic. The objective of this is to prevent re-establishment of non-desirable bacterial populations and thus help limit the re-occurrence of oral malodour over a prolonged period. In this paper, we discuss why contemporary probiotics are inadequate for treatment of oral malodour and examine the rationale for selection of particular bacterial species for future use in the treatment of this condition. In our preliminary trials of the use of a chlorhexidine rinse followed by strain K12 lozenges, the majority (8/13) of subjects with confirmed halitosis maintained reduced breath levels of volatile sulphur compounds for at least 2 weeks. We conclude that probiotic bacterial strains originally sourced from the indigenous oral microbiotas of healthy humans may have potential application as adjuncts for the prevention and treatment of halitosis.
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Keywords: halitosis, oral malodour, probiotic, *Streptococcus salivarius*

Introduction

Oral malodour is a problem which afflicts a large proportion of the adult population and it is one of the main reasons why many people are motivated to persist with regular oral hygiene. The most common oral malodour compounds are by-products of the metabolism of certain species of oral bacteria, mainly those on the dorsum of the tongue. A diverse consortium of gram-negative and gram-positive bacteria have been found to contribute to the problem and by contrast, certain bacterial species that predominate in the mouths of 'healthy' subjects become noticeably absent in subjects with halitosis (Kazor *et al*, 2003). Given that oral microorganisms, especially those on the tongue, are the primary cause of halitosis, current treatments focus on the use of chemical or physical antibacterial regimes to reduce the numbers of these bacteria. However, these treatments are typically of only short-term benefit, as the offensive bacteria quickly recover to former numbers after treatment is stopped.

Probiotics, defined by the World Health Organization as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host', may provide a supplementary treatment. Probiotics have been used to modulate bacterial populations in other regions of the human body either when it has been perceived that there is an 'imbalance' of the normal microbiota allowing unregulated growth of 'problematic' microorganisms or in order to replenish bacterial populations which play a specific role in a particular ecosystem. Some examples of successful applications of probiotics include the intestinal tract, where the occurrence of certain disease states has been reduced and the prevention of post antibiotic-associated diarrhoea (Reid *et al*, 2003), and in the vagina where they have been used to reduce the incidence of vaginosis and vaginitis (Reid *et al*, 2003).

Antimicrobial treatment indiscriminately depletes populations of both the problematic bacteria and those bacteria that are not thought to be implicated in halitosis, but which are likely to be important in the maintenance of a normal oral microenvironment. There are many factors that can influence the course of microbial succession and the ultimate restoration of a climax (balanced) ecosystem following exposure to broadly-active antimicrobials. Some bacteria are more resistant than others to the individual antimicrobial agents and these will repopulate the oral cavity more

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quickly when populations of their competitors have been depleted. The outcome of antimicrobial treatment is inevitably only a temporary reduction in malodour, until the halitosis-causing bacteria become re-established. Therefore, in order to achieve long-term reduction of malodour, either more targeted antimicrobials need to be applied that will minimize extermination of the beneficial microbiota, or specific replenishment of the microbiota with beneficial bacteria needs to take place shortly after antimicrobial therapy. The use of more specific antimicrobials is probably not feasible, given that there is a diverse array of microbial species already implicated in halitosis and undoubtedly more that are still requiring identification (Kazor *et al*, 2003). Preventing the re-growth of odour-causing organisms by pre-emptive colonization of the oral cavity with non-virulent, commensal microorganisms seems like a reasonable alternative.

Given the large number of internet sites dedicated to the sale of probiotic products to people with halitosis, one would anticipate that there are many well-substantiated scientific claims of the efficacy of these products. In reality, however, there are no properly-conducted clinical trials of probiotics in halitosis prevention. Unfortunately, many probiotic products either do not contain the microorganisms stipulated on the label or contain a microbial preparation of low viability (Huff, 2004). Our understanding of the mechanisms involved in probiotic action is in its infancy, but, it is already becoming apparent that replenishment is most efficacious with use of bacteria derived from a similar ecosystem to the proposed site of treatment (Colodner *et al*, 2003; Reid *et al*, 2003). Previous attempts to alleviate population imbalances at different sites using a single microorganism have not always been successful (Colodner *et al*, 2003). The phenotypic profile of probiotic bacteria is very important and validation of the key characteristics of probiotic strains must be established in properly controlled clinical trials conducted according to strict scientific criteria. To date, the principal applications of oral probiotics have been for the prevention of dental caries (Nase *et al*, 2001; Ahola *et al*, 2002; Comelli *et al*, 2002; Montalto *et al*, 2004). Dairy strains such as *Streptococcus thermophilus* and *Lactococcus lactis* have been investigated for their ability to weaken dental plaque biofilms (Comelli *et al*, 2002). Lactobacilli exist as commensals in the oral cavity, although they have a strong association with caries (Byun *et al*, 2004). Probiotic lactobacilli of intestinal origin have achieved only variable results in the reduction of *S. mutans* levels (Nase *et al*, 2001; Montalto *et al*, 2004). There is little if any literature on the use of probiotic microorganisms specifically isolated from the mouth for the maintenance of oral health. Given that all of the previously applied oral probiotics have been derived from the intestinal tract or elsewhere it seems that they may not be the most appropriate choice for halitosis prevention.

Indeed, a bacterium isolated from the oral cavity intuitively appears more likely to persist and to have characteristics important for establishing a stable oral microenvironment, especially if this bacterium is naturally predominant within the microbiotas of 'healthy' subjects. Given that the dorsum of the tongue is the origin of most malodour problems, a candidate probiotic to counter this condition should be able to persist within this particular ecosystem. Bacterial strains usually have affinity for particular tissues because of the presence of specific adhesive molecules on their cell surfaces (Reid and Burton, 2002). The production of anti-competitor molecules such as bacteriocins (protein antibiotics) also appears to confer an ecological advantage to some bacteria. When present in low concentrations they typically are signalling or inducer molecules, yet in higher concentrations they can exert antibiotic (killing) activity against their bacterial competitors. Therefore, production of bacteriocins by a bacterium can give it a useful advantage in highly competitive ecosystems. De Boever and Loesche (1995) suggested that the proteolytic microbiota colonizing the tongue makes the major contribution to halitosis through its action in degrading dietary and host proteins. Therefore, a probiotic strain that efficiently colonizes the tongue surface and does not produce odorous metabolic by-products would be highly advantageous.

Although there are very few in depth studies of the oral microbial populations of subjects with and without halitosis, some significant associations appear to be emerging. Previous culture-based studies have indicated that there is an increase in the ratio of aerobic to anaerobic bacteria after successful antimicrobial treatment and also an increase in *S. salivarius* cell counts (De Boever and Loesche, 1995). Also, a recent comprehensive 16S rDNA clone library study by Kazor *et al* (2003) showed that certain species were found specifically in individuals who were 'healthy' or in subjects having a 'diseased' state. *Streptococcus salivarius*, *Rothia mucilaginosa* and certain *Eubacterium* species were more frequently detected in 'healthy' subjects.

Streptococccus salivarius strains appear to be excellent candidates for an oral probiotic, since they are early colonizers of oral surfaces and are amongst the most numerically-predominant members of the tongue microbiota of 'healthy' individuals (Carlsson *et al*, 1970; Kazor *et al*, 2003). This species also has only a limited ability to produce volatile sulphur compounds (Yoshida *et al*, 2003) and is unlikely to contribute significantly to oral odour. *Streptococcus salivarius* has not been implicated either in caries or in other infectious diseases of humans and is most closely related to *S. thermophilus*, a bacterium widely used in the dairy food industry (formerly *S. salivarius* ssp. *thermophilus*). We have tested *S. salivarius* strains for production of a variety of characteristics relevant to their potential application as oral probiotics including: bacteriocins, persistence in the oral cavity, adhesion to various oral cells and viability on freeze drying and storage. *Streptococcus salivarius* strain K12 is considered to be a particularly good candidate, since it produces at least two lantibiotic-type bacteriocins which are especially potent against gram-positive bacteria. *Streptococcus salivarius* K12 (BLIS Technologies Ltd, Dunedin, New

Zealand) is currently marketed in New Zealand for the prevention of streptococcal sore throats.

In order to determine whether strain K12 might also be useful in the treatment of halitosis, a series of bacterial strains representative of species implicated in halitosis were tested to see if they were inhibited by the two bacteriocins produced by strain K12. Inhibition was observed of *S. anginosis*, *Eubacterium saburreum* and *Micromonas micros*, but not of *Porphymonas gingivalis* and *Prevotella intermedia* (Burton *et al*, 2004). On the other hand, when fresh saliva was inoculated onto agar medium impregnated with the bacteriocins produced by strain K12, inhibition of black-pigmented bacteria identified as *Prevotella* species was observed (J.P. Burton, C.N. Chilcott, C.J. Moore, unpublished data). In a preliminary study, a course of lozenges containing strain K12 were taken by 13 subjects with confirmed halitosis following mouth-rinsing with chlorhexidine. When measured after 1 week of using the K12 lozenges (4 days after ceasing use of the chlorhexidine), 11 of the subjects had volatile sulphur readings that were reduced by at least 100 ppb when compared with pretreatment levels. Eight subjects maintained substantially reduced VSC levels for at least 14 days (J.P. Burton, C.N. Chilcott, C.J. Moore, unpublished data). All subjects showed an increase in the levels of *S. salivarius* as a proportion of their total salivary populations and other measures of halitosis such as BANA reactivity and organoleptic scores were reduced (Burton *et al*, 2004).

Whilst the majority of the subjects tested in this pilot study had a favourable outcome, the mechanism(s) of volatile sulphur compound reduction was not clearly established. *Streptococcus salivarius* strain K12 does not appear to exhibit *in vitro* inhibition of all species implicated in halitosis and, therefore, other mechanisms of competition, such as saturation of attachment sites may have been influential. Antimicrobial treatments of polymicrobial diseases rarely target all of the bacteria implicated in the disease process, but the killing of some of the critical members of the consortia can cause disruption of the disease-associated population. Thus, the bacteriocins produced by strain K12 may have prevented re-growth of key microbial participants in the halitosis-associated ecosystem. Recent data from our laboratory also indicates that the bacteriocins produced by strain K12 are auto-inducible and that they can cross-stimulate bacteriocin production by other indigenous *S. salivarius* and related species. Thus, the administration of strain K12 may also have boosted the production levels of these antibacterial compounds by the subjects' existing oral microbiota.

In this article, we have briefly explored the rationale for use of probiotics in the prevention of halitosis. Consideration has been given to the nature of currently available oral probiotics and to the importance of strain selection and careful phenotypic characterization. We believe that competitive *S. salivarius* strains have great potential for the control of halitosis and for the prevention of a variety of oral bacterial infections. Whilst further double-blind, placebo-controlled studies are clearly necessary, the outcome of our pilot study encourages us to further investigate *S. salivarius* strains as a novel adjunct for the treatment of halitosis.

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ORIGINAL ARTICLE

Pilot study of a breath test for volatile organic compounds associated with oral malodor: evidence for the role of oxidative stress

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BACKGROUND: We performed a pilot study of a new method to identify the volatile organic compounds (VOCs) in breath associated with oral malodor, using gas chromatography and mass spectroscopy (GC/MS).
METHODS: Oral cavity breath was collected from seven patients with oral malodor. Breath samples (150 ml) were concentrated onto sorbent traps and analyzed by GC/MS.
RESULTS: Organoleptic scores ranged from 3.0 to 4.0 (mean = 3.3) on a scale of 0–5. Twenty-four of 30 (80.0%) of the most abundant oral malodor volatile organic compounds (OMVOCs) were alkanes and methylated alkanes. These VOCs are products of oxidative stress, generated by lipid peroxidation of polyunsaturated fatty acids in cell membranes.
CONCLUSIONS: Increased oxidative stress in the oral cavity of patients with oral malodor may account for the increased risk of atherosclerosis, coronary heart disease and stroke associated with periodontal disease. The breath test for OMVOCs could potentially provide an objective new test for the assessment of oral malodor.
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Keywords: volatile organic compounds; GCMS; oral malodor; breath test

Introduction

Oral malodor is a common disorder, often associated with bacterial infections of the periodontal area and the dorsum of the tongue (Figueiredo *et al*, 2002). The intensity of oral malodor may be ranked by expert odor judges employing an organoleptic scale, or with assays of volatile organic compounds (VOCs) in breath, e.g. sulfur-containing compounds (Scully *et al*, 1997). We report here the findings of a sensitive new method employing gas chromatography and mass spectroscopy (GC/MS) for detecting a wide spectrum of oral malodor volatile organic compounds (OMVOCs). This was a pilot study focused principally on methods development, in order to evaluate the feasibility of a new analytical method prior to evaluating it in a larger scale study.

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Materials and methods

Human subjects
Seven subjects were recruited with a history of oral malodor. Two trained organoleptic judges independently scored their severity of oral malodor using the following scale: 0, no odor present; 1, barely noticeable odor; 2, slight but clearly noticeable odor; 3, moderate odor; 4, strong odor; 5, extremely strong odor. Human research was approved by the Institutional Review Board of Saint Vincent Catholic Medical Centers, New York, NY, USA and the Biomedical Institute of America, San Diego, CA, USA.

Breath test for OMVOCs
All subjects donated a sample of oral cavity breath by inflating a multi-laminate 200 ml breath collection bag (Quintron, Inc., Milwaukee, WI, USA). Bags were heated to 38°C, and breath samples (150 ml) were extracted and injected onto sorbent traps (Carbotrap, Supelco, Bellefonte, PA, USA) in order to capture VOCs. Samples were assayed by GC/MS using a reported method (Phillips *et al*, 1999). VOCs were identified by a computer-based library and quantified by their ratio to an internal standard. Background VOC contaminants in the breath collection bags were determined by similar assays of bags inflated with ultra-pure helium, and subtracted from VOCs in breath.

Results

Organoleptic scores ranged from 3.0 to 4.0, mean = 3.3. The 30 most abundant VOCs in oral cavity breath are shown in Table 1. An unexpected finding was that the majority of these VOCs (24/30, 80%) were alkanes or alkane derivatives (displayed in Figure 1).

Table 1 Most abundant VOCs in breath of patients with oral malodor

Breath VOC	*Mean*
Benzene, methyl-	8.25
Butane, 2,2,3,3-tetramethyl-	4.75
Ethanol	4.64
Hexane, 2,2,5-trimethyl-	4.09
1-Propene, 2-methyl-	4
1,3-Butadiene, 2-methyl-	3.87
Nonane, 3-methyl-5-propyl-	3.59
Decane, 2,2-dimethyl-	3.42
Hexane, 3-methyl-	3.18
Cyclopentane, methyl-	2.94
Hexane	2.84
Cyclohexane, methyl-	2.63
Hexane, 2-methyl-	2.63
Cyclohexane	2
Pentane, 2,3-dimethyl-	1.5
Undecane, 3-methyl-	1.38
Butane, 2-methyl-	1.21
2-Butanone	1.11
Pentane, 3-methyl-	1.01
Heptane	0.96
Pentane, 3-ethyl-2,2-dimethyl-	0.82
Decane, 2,2,8-trimethyl-	0.75
Pentane, 2,3,3-trimethyl-	0.75
Pentane, 2-methyl-	0.75
Pentane, 2,3,4-trimethyl-	0.72
Hexane, 2,2,4-trimethyl-	0.57
Pentane	0.55
Acetaldehyde	0.55
Cyclopentane, ethyl-	0.54
Hexane, 2,2,3-trimethyl-	0.53

The 30 most abundant VOCs are ranked by relative abundance (mean value of ratio to abundance of an internal standard). VOCs shown in bold script are alkanes or methylated alkanes.

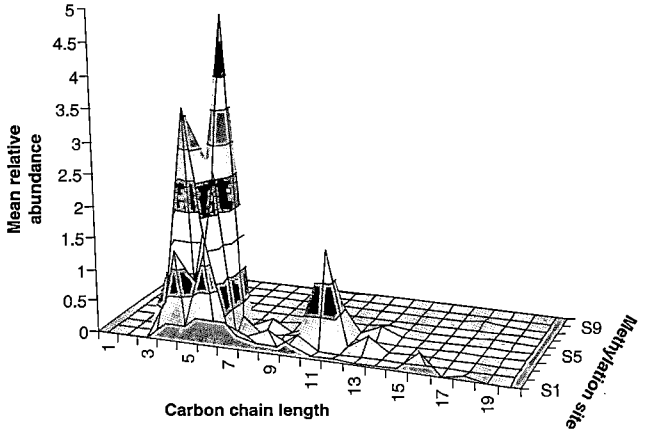

Figure 1 Oral malodor volatile organic compounds (OMVOCs) generated by oxidative stress. This figure displays the mean abundance of alkanes and monomethylated alkanes in the oral cavity breath of seven subjects with oral malodor. These VOCs are products of oxidative stress which are generated by lipid peroxidation of membrane polyunsaturated fatty acids. The carbon chain length is shown on the *x*-axis, ranging from C4 (butane) to C20 (eicosane), and the methylation site is shown on the *z*-axis. The abundance is shown on the *y*-axis

Discussion

We have previously reported the composition of VOCs in alveolar breath employing a different collection method, but a similar analytical method (Phillips *et al*, 1999). A study of 50 normal subjects revealed approximately 200 VOCs in each sample of breath, and more than 3000 different VOCs in the entire group. A very wide spectrum of chemical compounds was observed in alveolar breath, and it is possible that oral breath may also harbor a similar diversity of VOCs. However, one limitation of the collection method is that the adsorptive material in the sorbent trap selectively captures VOCs with two carbon atoms or more, so it is possible that lower molecular weight VOCs (e.g. sulfur compounds such as H_2S) may be underestimated in this assay.

The alkanes and alkane derivatives observed in oral cavity breath are known to be products of oxidative stress, in which mitochondria produce excessive quantities of reactive oxygen species that leak into the cytoplasm and oxidize several biologically important molecules, including DNA, lipids, carbohydrates and proteins (Therond *et al*, 2000). Lipid peroxidation of polyunsaturated fatty acids generates peroxyl radical which decomposes to aldehydes and alkanes (Dotan *et al*, 2004; Kneepkens *et al*, 1992). The abundance of volatile alkanes and methylated alkanes in the breath varies with the intensity of oxidative stress (Phillips *et al*, 2000).

Increased oxidative stress in the oral cavity of patients with oral malodor carries important clinical implications. Oral malodor is usually a consequence of infection in the oral cavity (Bosy, 1997), and periodontal infection has been linked with increased oxidative stress (Sculley and Langley-Evans, 2003; Takane *et al*, 2002). Periodontal disease has also been linked with an increased risk of atherosclerosis (Haynes and Stanford, 2003), coronary heart disease (Lopez *et al*, 2002) and stroke (Grau *et al*, 2004). These observations may be causally linked: it is possible that a focus of oral infection (e.g. gingivitis or periodontitis) generates increased oxidative stress, resulting in increased oxidation of LDL-cholesterol and accelerated atherosclerosis, thereby increasing the risk of coronary heart disease and stroke (Weinbrenner *et al*, 2003). The abundance of these VOC markers of oxidative stress was dramatically higher on oral breath than in previous observations of alveolar breath in normal subjects. However, correlation of oxidative stress with the intensity of oral malodor will require a larger future study that permits comparison of subjects with high and low levels of oral malodor.

These findings suggest that oral malodor may be considerably more serious than a social embarrassment – it may also be a sign of increased oxidative stress in the oral cavity and carry an increased risk of life-threatening vascular disease. This test for OMVOCs could potentially provide an objective new test for the assessment of oral malodor, and a larger clinical study is in progress to test this hypothesis.

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ORIGINAL ARTICLE

Clinical assessment of oral malodor intensity expressed as absolute value using an electronic nose

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OBJECTIVES: In our previous study, scores determined via a multiple linear regression method (EN-MLR) involving an electronic nose provided objective halitosis-related measurements; however, this model afforded only relative expression exclusively. The objective of this investigation was to assess clinically oral malodor intensity expressed as an absolute value using an electronic nose.

SUBJECTS AND METHODS: Sixty-six subjects were evaluated based on results of an actual organoleptic test (OLT), measurements of volatile sulfur compound (VSC) concentrations, a score representing malodor intensity (EN-MI) as the absolute value and EN-MLR measured with an electronic nose system. Oral health parameters were also examined.

RESULTS: The OLT score served as a benchmark. The area under the receiver-operating characteristic (ROC) plots of EN-MI score (0.975) was significantly larger than that of log VSC (0.896) ($P = 0.036$); however, the area did not differ significantly from that of EN-MLR score (0.932). Percentage of teeth with pocket depth ≥4 mm, tongue coating score and plaque control record displayed meaningful association with EN-MI score in multiple logistic regression analyses.

CONCLUSION: Oral malodor intensity expressed as an absolute value employing an electronic nose may be a suitable method for clinical evaluation of oral malodor.
Oral Diseases (2005) **11** (Suppl. 1), 35–36

Keywords: oral malodor; clinical assessment; electronic nose; oral health parameters

Introduction

The two primary methods applicable to clinical analysis of oral malodor are organoleptic test (OLT) and instrumental analysis, such as gas chromatography or use of a portable sulfide monitor (Rosenberg *et al*, 1991). However, since both techniques exhibit disadvantages, there remains no ideal objective approach for clinical assessment of the degree of oral malodor (Rosenberg, 1996). We previously reported that the scores determined with an electronic nose under top-note mode, provided objective halitosis-related measurements. However, those data were obtained via the multiple linear regression method (EN-MLR) and yielded relative rather than absolute values (Tanaka *et al*, 2004a). This investigation attempted to assess clinically oral malodor intensity expressed as an absolute value utilizing an electronic nose.

Subjects and methods

The subject population consisted of 46 individuals (mean age, 40.8 years) characterized by odor-judge ratings of ≥2.0 and 20 participants (mean age, 41.6 years) with odor-judge ratings of <2.0. Breath malodor was assessed with an electronic nose. Furthermore, volatile sulfur compound (VSC) measurements were conducted on a gas chromatograph; additionally, an OLT was performed.

The FF-1 odor discrimination analyzer (electronic nose, Shimadzu Ltd, Kyoto, Japan) utilized in the present study consisted of a preconcentrator, an array of six metal oxide semiconductor sensors selected for their distinct sensitivity and selectivity to fragrant substances, and pattern recognition software. The electronic nose was calibrated with nine standard gases including hydrogen sulfide, methylmercaptan, ammonia, trimethylamine, propionic acid, butylaldehyde, butylacetate, toluene and heptane (Shimadzu Ltd) before the measurement. The gas sample was introduced into the trap tube for 30 s, and then the trapped odor was driven to the sensor section with pure nitrogen. The odor spectrum was determined for nine categories of gases in the odor space formed by multiple output signals of sensors. The malodor intensity of sample gases as an absolute value was expressed as the vectorial sum of the malodor intensity of each category of gas (Kita *et al*, 2000). The score as a relative value was also obtained via a multiple linear regression method (Tanaka *et al*, 2004a). Probing depth was measured at two points

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around each tooth in all subjects and periodontal status was determined as a percentage of teeth displaying probing depth of ≥4 mm. O'Leary's plaque control records were also employed to assess oral hygiene status. In addition, tongue coating scores were determined by multiplying thickness by area (Tanaka *et al*, 2003).

Statistical comparisons of EN-MI, EN-MLR score and VSC level were analyzed using receiver-operating characteristic (ROC) plots with Rockit 0.9B Beta Version. Multiple linear regression and logistic regression analyses served to identify those oral health variables demonstrating a significant independent effect on EN-MI scores.

Results

Correlations between actual OLT score and EN-MI, EN-MLR scores or log total VSC derived from gas chromatography were examined. These three correlation coefficients were 0.81, 0.78 and 0.73, respectively. No meaningful difference was observed among the three correlation coefficients. ROC sensitivity-specificity plots afforded an appropriate protocol for comparison of EN-MI, EN-MLR scores and log total VSC data for classification of subjects with or without actual OLT score ≥2.0. Area under the ROC plots representing EN-MI scores (0.975) was markedly larger than that of log VSC (0.896) ($P = 0.036$); however, it did not differ significantly from that of EN-MLR (0.932) (Figure 1).

Logistic regression analyses were conducted which corresponded to the possibility of placement of subjects in the upper 25th percentile of EN-MI score distribution as a dependent variable. Percentage of teeth exhibiting probing depth of ≥4 mm, tongue coating score and plaque control record displayed significant association with EN-MI scores (odds ratios of 13.0, 7.1 and 28.2, respectively) ($P < 0.05$).

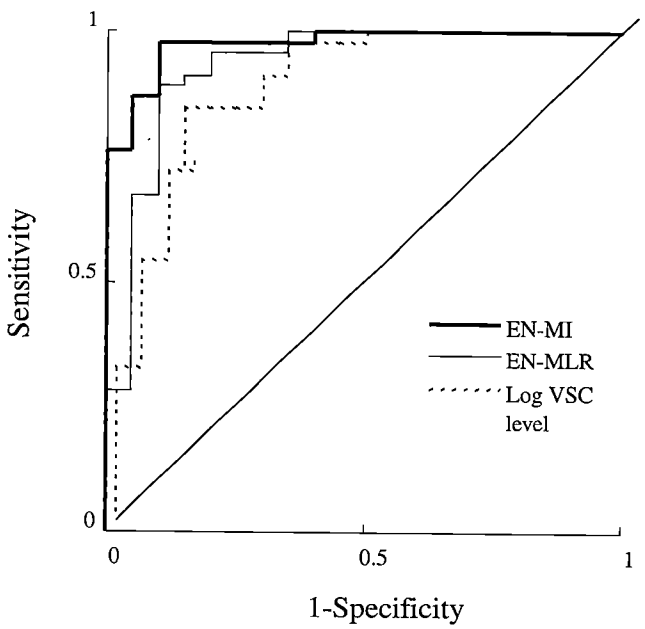

Figure 1 ROC plots comparing EN-MI, EN-MLR and log VSC data utilized for classification of subjects with or without OLT score of ≥2.0. Areas under ROC plots were 0.975 (EN-MI), 0.932 (EN-MLR) and 0.896 (log VSC)

Discussion

In the present investigation, EN-MI scores were compared with EN-MLR scores as oral malodor assessment employing an electronic nose system. The correlation coefficient between EN-MI score and OLT score ($r = 0.81$) was nearly identical to that between EN-MLR and OLT score ($r = 0.78$). Area under the ROC curve representing EM-MI score was also similar to that of EN-MLR; in contrast, it was significantly larger than that of log VSC. This finding suggests that EN-MI score determined with the electronic nose demonstrated a level of accuracy higher than that of log VSC for classification of subjects with or without actual OLT score of ≥2.0.

This study also established that EN-MI scores were independently correlated with periodontal health status, tongue coating and supra-gingival plaque accumulation. These results were consistent with those of EN-MLR scores (Tanaka *et al*, 2004a). We also previously reported that the periodontal pathogens on tongue dorsa contributed markedly to VSC production (Tanaka *et al*, 2004b). However, since the proportions of periodontal pathogens were weakly associated with OLT score, we suggested that other malodorous compounds in addition to VSC might also be related to OLT scores (Tanaka *et al*, 2004b). EN-MI may be attributed to various malodorous compounds which may be produced mainly at supra-gingival plaque, periodontal pocket and tongue coating. Thus, the relationship between amount of supra-gingival plaque, periodontal pocket depth and tongue coating and oral malodor may be effectively detected by EN-MI. We conclude that the absolute expression of malodor intensity with an electronic nose can afford objective halitosis-related measurements.

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ORIGINAL ARTICLE

Comparison of ninhydrin method of detecting amine compounds with other methods of halitosis detection

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OBJECTIVES: Halitosis is a result of overactivity of Gram-negative bacteria for which protein amino acids are the major source of energy. Therefore, statistical correlation between concentrations of volatile sulphur compounds (VSCs) and low-molecular-weight amines should be expected.

MATERIALS AND METHODS: Eighty-four patients suffering from halitosis and 40 healthy volunteers aged 20–62 years (average 39.7) participated in the study. In all subjects low-molecular-weight amines were evaluated by the ninhydrin method. Patients with halitosis were randomized into treatment groups. Zinc tablets, tablets and mouthwash containing chlorhexidine or lyophilized lactic acid-forming bacteria were used.

RESULTS: Analysis showed that the level of amines was highest in subjects with halitosis (0.39, s.d. ± 0.06, $P < 0.001$) and correlated significantly with results of VSC measurement and organoleptic scores ($P < 0.001$). Reduction of amine levels after treatment was statistically significant ($P < 0.001$). Three months following treatment the amine levels began to increase (0.37, s.d. ± 0.05). The amine levels in healthy controls were lower (0.29, s.d. ± 0.07) and remained at a stable level.

CONCLUSION: The salivary amine levels significantly correlated with VSC levels and organoleptic scores. Therefore, the ninhydrin method of detecting salivary amines may be an alternative or additional method of diagnosing halitosis. This method may also be used to evaluate treatment efficacy.

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Keywords: halitosis; metabolism of amino acids and peptides; saliva/analysis; volatile/analysis

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Introduction

Patients presenting with halitosis expect effective diagnosis and treatment from dental practitioners. Therefore rapid, simple and relatively inexpensive methods to diagnose halitosis and control its treatment are necessary. Such a possibility is given by using a portable sulphide monitor to measure levels of volatile sulphur compounds (VSC) (Rosenberg *et al*, 1991). Unfortunately some of the components of oral malodour, e.g. low-molecular-weight amines cannot be measured using this method. Gases which are components of oral malodour originate from the breakdown of peptides and glycopeptides by bacterial putrefaction in the oral cavity. During this process peptides are hydrolysed to amino acids which further are metabolized to amines or polyamines (lysine, arginine and ornithine) (Patocka and Kuehn, 2000). Correlations between malodour levels and diamines in saliva have been found previously (Goldberg *et al*, 1994) and Klineberg and Codipilly (1995) have shown elevated odour levels after addition of lysine to cultures of oral anaerobes. The ninhydrin colorimetric reaction is a simple, rapid and inexpensive method which may be used for examination of amino acids and low-molecular-weight amines. Detection of low-molecular-weight amines will give information about the level of substrates used during bacterial putrefaction. In theory, the level of the bacterial putrefaction substrates should be important as an aetiologic factor in halitosis.

The aim of the study was to evaluate statistical correlation between the concentration of volatile sulphur-containing compounds, organoleptic measurement and low-molecular-weight amines.

Materials and methods

The study was performed on patients who visited the Department of Conservative Dentistry in Warsaw, Poland. Eighty-four subjects suffering from oral halitosis diagnosed at the beginning of the study (mean age 41.2 ± 11.94, range from 20 to 62; 38.1% males, 61.9%

females) and 40 healthy subjects (mean age 36.8 ± 11.74, range from 22 to 57; 27.5% males, 72.5% females) were recruited for participation in the study. Halitosis was diagnosed if the average level of VSCs was ≥125 ppb and organoleptic measurement was ≥2. Healthy subjects' average VSC level was ≤75 ppb and organoleptic measurement was close to zero. Subjects did not report any systemic or periodontal disorders. No one had a history of periodontal treatment or antibiotic medications during the previous 3 months. Patients suffering from halitosis were randomized into four test groups: A, 25 persons; B, 25 persons; C, 20 persons; D, 14 persons. Group A was treated three times a day with 7 mg zinc tablets for 21 days. Group B was given 0.1% chlorhexidine digluconate mouthwash in 30 s rinses three times a day and group C 2.0 mg chlorhexidine digluconate tablets four times a day for 2 weeks. Group D received three tablets containing lyophilized lactic acid-forming bacteria once a day after breakfast for 7 days – powder was applied under the tongue. Examination of VSC, organoleptic measurement and saliva analysis was performed for subjects from the test groups on day 0 (before treatment), 7, 14 and 21 and after 3 months and for controls on day 0, 21 and after 3 months. Examinations were performed by three independent examiners. Organoleptic measurement was always performed in the morning before breakfast. Subjects exhaled into a single-use 10 cm plastic tube. The level of oral malodour was evaluated on a 0 to 5 scale as follows: 0 = absence of odour, 1 = questionable odour, 2 = slight malodour, 3 = moderate malodour, 4 = strong malodour, 5 = severe malodour (Rosenberg *et al*, 1991). Volatile sulphide measurements were performed using a portable hydrogen sulphide monitor (Halimeter RH-17K, Interscan Corp., Chatsworth, CA, USA). Subjects were examined in the morning, before organoleptic measurement. The single-use plastic tube was connected to the air inlet of the monitor. Patients were asked to put the other side of this tube in to the oral cavity and to breathe through the nose during the examination. Maximum and average values from three measurements of VSC were obtained in parts per billion (ppb). Data were collected and visualized on the computer using special software (HaliSoft v.1.0e, Ansyco GmbH, Ostring, Germany). Total amine level in saliva was determined with ninhydrin colorimetric reaction (Moore and Stein, 1948; Amato and Ladd, 1994) with some modification. Briefly, sample of saliva (100 μl) and isopropanol (100 μl) was mixed and centrifuged. The supernatant (100 μl) was diluted with isopropanol (400 μl), buffer solution pH 5 (1.5 ml) and ninhydrin reagent (2 ml). The mixture was refluxed in a water bath for 30 min, cooling to 21°C and diluted with isopropanol to a total volume of 10 ml. Light absorbance readings were determined at 570 nm using a MK6/6 Carl Zeiss (Jena, Germany) spectrometer. Resting saliva was taken for re-examination and archival storage at −20°C. Statistical analysis was performed using Wilcoxon's test. Spearman rank correlation was used to determine the relationship between organoleptic scores, VSC and amine levels.

Results

Analysis showed that the levels of amines were highest in subjects with halitosis (0.39, s.d. ± 0.06, $P < 0.001$) (Table 1) and correlated significantly with results of VSC measurement and organoleptic scores ($P < 0.001$) (Table 2). Reduction of salivary amine levels after treatment were statistically significant in all test groups except those using 0.1% chlorhexidine digluconate mouthwash (Figure 1). After 3 months the amine levels began to increase (0.37, s.d. ± 0.05). The amine levels in healthy controls were lower (0.29, s.d. ± 0.07) and remained at a stable level. Table 3 shows organoleptic improvement after treatment in all test groups and changes in VSC levels. Although the levels of VSC were lower in all groups, changes after treatment with tablets containing lyophilized lactic acid-forming bacteria were not statistically significant.

Discussion

A method of diagnosing halitosis using a combination of organoleptic method and measurement of VSC levels using a halimeter has been shown to be effective and confirmed by many workers. These diagnostic methods are fast, simple and inexpensive. Correlations between VSC levels and organoleptic scores in the present study were comparable with those previously obtained by Rosenberg *et al* (1991). The examination of polyamines is complicated; therefore most often analyses are limited to detection of putrescine and cadaverine. Usually high-performance liquid chromatography is used for detection of polyamines. Because this is relatively expensive and time consuming it cannot be used for examination of a large subject population (Goldberg *et al*, 1994). The ninhydrin colorimetric reaction is widely used for detecting amino acids and low-molecular-weight amines using a classical amine analyser, e.g. for checking amine levels in the blood. It was therefore decided to use this simple method in this study to determine amine levels in the saliva. Amine levels were higher in the saliva of subjects suffering from halitosis and lower in healthy controls. As well, amine levels correlated significantly

Table 1 Mean amine levels of halitosis and healthy subjects measured in extinction (E)

	Amine levels ± s.d.				
	Day 0	*Day 7*	*Day 14*	*Day 21*	*3 months*
Halitosis	0.389 ± 0.06	0.361 ± 0.07	0.362 ± 0.06	0.366 ± 0.06	0.371 ± 0.05
Healthy	0.285 ± 0.05			0.289 ± 0.06	0.292 ± 0.06

Table 2 Correlations between volatile sulphur compound (VSC) levels, organoleptic measurements and amine levels

Examination	*VSC*	*Organoleptic*
Amine levels	$r = 0.5389$; $P < 0.001$	$r = 0.5982$; $P < 0.001$
VSC		$r = 0.7820$; $P < 0.001$

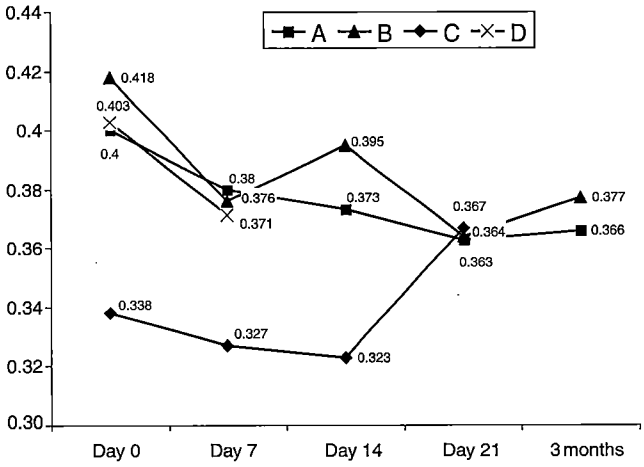

Figure 1 Mean amine levels in treatment groups – 3-month observation. A = three times a day 7 mg zinc tablets for 21 days. B = 0.1% chlorhexidine digluconate mouthwash in 30 s rinses two times a day. C = 2.0 mg chlorhexidine digluconate tablets four times a day for 2 weeks. D = three tablets containing lyophilized lactic acid-forming bacteria once a day after breakfast for 7 days – powder was applied under the tongue

Table 3 Organoleptic and volatile sulphur compound (VSC) changes after treatment in all test groups

	Mean organoleptic score ± s.d.		*Mean VSC ± s.d.*	
	Day 0	*After treatment*	*Day 0*	*After treatment*
A	3.40 ± 0.65	1.79 ± 0.88	277.40 ± 215.17	123.04 ± 62.14
B	3.00 ± 0.76	1.87 ± 0.92	255.08 ± 185.52	202.25 ± 111.71
C	3.70 ± 1.30	2.30 ± 0.86	319.75 ± 219.08	166.55 ± 92.25
D	3.79 ± 0.89	2.50 ± 1.65	295.29 ± 191.77	210.71 ± 97.76
K	0	0	60.08 ± 12.90	57.05 ± 12.86

A = three times a day 7 mg zinc tablets for 21 days.
B = 0.1% chlorhexidine digluconate mouthwash in 30 s rinses two times a day.
C = 2.0 mg chlorhexidine digluconate tablets four times a day for 2 weeks.
D = three tablets containing lyophilized lactic acid-forming bacteria once a day after breakfast for 7 days – powder was applied under the tongue.
K = healthy controls – no treatment.

with results of VSC measurement and organoleptic scores before treatment. Moreover, amine, VSC levels and organoleptic scores were lower after treatment of halitosis in all groups. Changes in VSC levels and organoleptic measurements were similar to those observed in other studies (Rolla *et al*, 2002; Rosing *et al*, 2002; Young *et al*, 2003). This suggests that in the oral cavity of subjects with halitosis the level of substrates used to produce oral malodour is higher and may be controlled. The ninhydrin colorimetric reaction was helpful in the indication of malodour substrate quantity in general, as well as concentrations of amines in particular.

In conclusion, the salivary amine levels significantly correlated with VSC levels measured by halimeter and organoleptic scores. Therefore, the ninhydrin method of detecting salivary amines may be an alternative method of diagnosing halitosis. This method may also be used to evaluate treatment efficacy.

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ORIGINAL ARTICLE

Effects of training on odor judges scoring intensity

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OBJECTIVE: This pilot study was intended to test whether a training protocol improved validity of odor judges (OJs), with or without experience, and whether odorant types differed in error proneness.

METHODS: The OJs (four experienced, two inexperienced) completed a 4-phase training protocol based on the American Society of Testing and Materials standards (ASTM): (i) introduction to sensory scales, n-butanol reference, sniffing techniques; (ii) pretraining measurements; 20 samples of varying intensities of four unpleasant and three pleasant odorants; (iii) exercises assessing quality, intensity, ranking, and matching; and (iv) post-training measurements.

MAIN OUTCOME MEASURES: Subjects' intensity scores were analyzed as the absolute difference from the 'true' intensity (ASTM n-butanol standard) using repeated measures ANOVA.

RESULTS: Training significantly ($P = 0.02$) reduced OJ errors. Experienced and novice judges did not differ in average errors ($P = 0.99$), or in improvement in error from pre- to post-training ($P = 0.94$). Improvement was consistent from pre- to post-training for all odorants except dimethylsulfide for which errors worsened ($P = 0.01$). Unpleasant and pleasant odorants differed ($P = 0.006$) in error. After removing water the effects of water control scores from the pleasant odorants, the difference was not significant ($P = 0.26$).

CONCLUSIONS: The OJs improved in their ability to assess odor intensity irrespective of previous experience. Training is recommended for all OJs prior to research trials.

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Keywords: oral malodor; sensory panels; organoleptic judges; odorants; training

Introduction

Research reports the major offensive odorants responsible for oral malodor (MO) are the volatile sulfur compounds (VSCs) hydrogen sulfide, dimethylsulfide (DMS), and methylmercaptan (Tonzetich, 1971; Schmidt *et al*, 1978; Kostelc *et al*, 1984). The current instruments used to assess oral malodor are the gas chromatograph (GC), sulfide monitors (Halimeter, OralChroma, Brethtron, etc.), and sensory odor judges (OJs). The GC and sulfide monitors quantify and in some cases identify sulfur compounds in concentrations as low as a few p.p.b. (Tonzetich, 1971; Schmidt *et al*, 1978; Rosenberg *et al*, 1991a,b; Yaegaki, 2002; Lenton *et al*, 2004). However, these sensors are only useful for identifying VSCs.

Sensory OJs can not only detect and recognize compounds but also can discriminate complex mixtures, so OJs are considered the gold standard for oral malodor assessment. OJs assess odors for intensity or offensiveness (Allison and Katz, 1919; Rosenberg *et al*, 2001a,b) and for hedonic tone (pleasantness) (Chambers and Wolf, 1996). Currently, the American Dental Association requires that for clinical trials, intensity or hedonic assessments be performed by at least two trained and calibrated OJs blinded to each other (ADA Council on Scientific Affairs, 2003).

Although training and calibration of sensory OJs has been well-documented in other disciplines (foods/beverages, air/water quality, personal care products), few data have been published on methods for training oral malodor judges. To date, most academic groups involved in oral malodor research have trained directly with malodor subjects. However, industry uses training protocols based on American Society for Testing and Materials (ASTM) standards. However, these studies are rarely published due to the proprietary nature of the business. The objective of this pilot study was to test if training OJs, experience in MO assessment and types of odorants improved OJ's intensity scores.

Methods and materials

Subject population

The subject population consisted of four sensory OJs with experience in assessing MO and two without experience. OJs participated in a 4-phase training protocol: (i) introduction to sensory techniques; (ii) pretraining; (iii) sensory training exercises; and (iv) post-training. The training protocol was adapted from the Wheeler *et al* (1981) Guidelines for the Selection, and Training of Sensory Panel Members, Chambers and

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Wolf (1996) Sensory Testing Methods, Subcommittee E 18.04 on Fundamentals of Sensory (1999) Standard Practices for Referencing Supra threshold Odor Intensity, and Sensory Evaluation Techniques (Meilgaard *et al*, 1999a).

Introduction to sensory techniques
The OJs received demonstrations on handling samples and sniffing techniques and were briefed on using the odor intensity scale (Table 1A), hedonic tone scale (Table 1B) and standard odor descriptors (McGinley and McGinley, 2002). OJs received a sample reference odorant (n-butanol) that had been determined by study investigators to have an intensity level of 3 for oral malodor. OJs were allowed to sniff and become familiar with the n-butanol intensity level 3.

Pretraining OJ measurements
The OJs independently assessed 20 odorant samples for odor hedonic tone, quality, and intensity. Each sample contained approximately 30 ml of liquid odorant in a 60 ml glass amber bottle with a screw cap. Samples consisted of seven different odorants, one neutral (water), four unpleasant (skatole, putrescine, DMS, and butyric acid) and two pleasant (jasmine, orange) of varying intensities. Study investigators assigned each odorant sample a 'true' intensity score by reference to the 5-point n-butanol study scale (Table 2), adapted from the ASTM 10-point n-butanol scale. OJs assessed odorants at 3-min intervals and were allowed to compare samples to the reference n-butanol, level 3, as needed.

Sensory training exercises
Training consisted of a series of didactic and laboratory practice sessions. Didactic sessions included discussions on the purpose of sensory training, influence of emotional factors, chain of sensory perception (stimulus → sensation and detection → perception and recognition → decision response) and odorant scales, qualities and descriptors. OJ preparation, reducing bias, adaptation and habituation were also discussed. Laboratory sessions included exercises on assessing descriptive qualities, detection and discrimination of similar compounds, memory matching of identical and similar odorants, odorant intensity ranking and relating odorants to the n-butanol reference scale. All laboratory exercises included training, practice, discussion and reassessment of skills. A variety of pleasant (jasmine, orange, pineapple, peppermint, rose, cinnamon, eucalyptus, lemon), unpleasant odorants (garlic, alcohol, hydrogen sulfide, skatole, putrescine, butyric acid, DMS) and neutral odorants (water, pine, spearmint, vanilla, apple, pear) with varying intensities were used during the training exercises.

Post-training OJ measurements
The same 20 samples assessed during pretraining were assessed for the post-training measurement, but in a different random order. OJs assessed samples at 3-min intervals and received a freshly mixed bottle of n-butanol of intensity level 3.

Statistical analysis
Statistical analysis used repeated measures ANOVA, where a 'subject' was an OJ. The dependent variable was the absolute difference between a judge's score and the truth as listed in Table 3 (the absolute difference is the difference with negative signs removed). The fixed effects were experienced *vs* non-experienced, pre- *vs* post-training, gas, and their interactions. A preselected comparison (a 'contrast') was made between pleasant and unpleasant gases using the gas as main effect. Standard errors are derived using error terms from the ANOVA.

Table 1 Descriptors for the organoleptic odor intensity and hedonic scales

(A) Odor intensity scale	*(B) Hedonic scale*
1 = no perceived odor	+2 = like very much
2 = faint odor noticed	+1 = like
3 = moderate odor	0 = do not like or dislike
4 = strong odor	−1 = dislike
5 = extremely strong odor	−2 = dislike very much

Table 2 ASTM and study scale relationships to butanol concentrations (p.p.m.)

ASTM 10-point scale (n-butanol)	*Study scale (n-butanol)*
1 = 12	1 = 25
2 = 24	2 = 225
3 = 48	3 = 675
4 = 96	4 = 2025
5 = 194	5 = 6075
6 = 388	
7 = 775	
8 = 1550	
9 = 3100	
10 = 6200	

Results

Overall, training significantly reduced (improved) OJ errors (Table 3, item 1; $P = 0.02$). Previous experience

Table 3 Average absolute error for selected groups of measurements, pre- and post-training

	Average absolute error		
	Pretraining	*Post-training*	*s.e. of average absolute error*[a]
Overall	1.13	0.77	0.09
Experienced	1.13	0.76	0.15
No experience	1.13	0.78	0.10
Butyric acid	1.33	0.83	0.23
Citrus (orange)	1.29	0.53	0.19
Dimethylsulfide	0.97	1.58	0.23
Water	0.42	0.08	0.23
Floral (jasmine)	1.24	0.61	0.19
Putrescine	1.33	0.81	0.19
Skatole	1.11	0.72	0.19

[a]Applies to both pre- and post-testing.

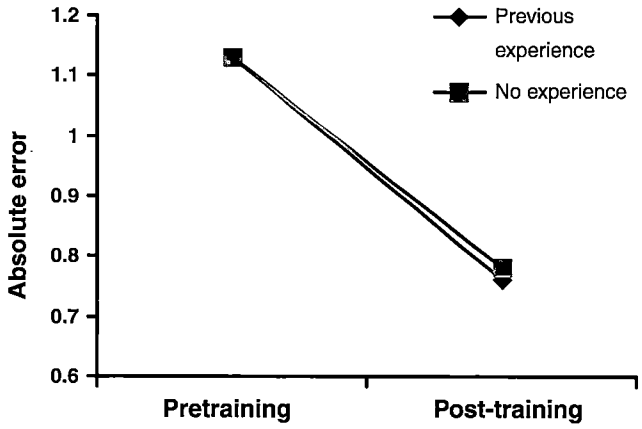

Figure 1 Relationship between odor judges previous experience and improvement (reduction in errors) from pre- to post-training, for all odorants combined

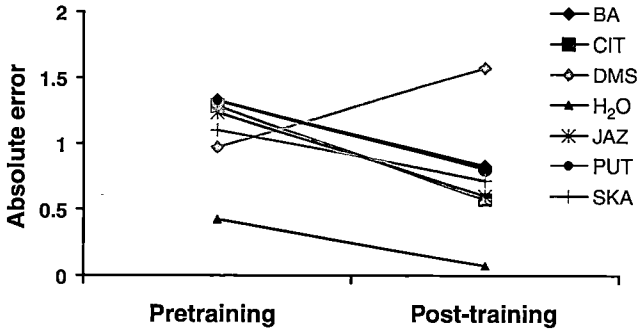

Figure 2 Average pre- and post-training odor judge scores for each odorant

was not associated with the size of the errors (experienced 0.94, no experience 0.95; $P = 0.99$). Experience was also not associated with the improvement (reduction in errors) from pre- to post-training (Figure 1; Table 3, item 2; $P = 0.84$).

Judges improved from pre- to post-training for all odorants except DMS (gas-by-training interaction, $P = 0.01$), for which they worsened (Figure 2; Table 3, item 3). Judges tended to have greater errors scoring unpleasant odorants (unpleasant 1.09, pleasant 0.70; $P = 0.006$). However, after removing the water score from the pleasant odorants, the difference was no longer significant (pleasant without water 0.93; $P = 0.26$). Figure 2 shows why; water had much lower errors both pre- and post-training than any other compound.

Discussion

Sensory odor assessment attempts to measure, analyze, and interpret the olfactory system's reaction to a perceived odor. It is difficult to assess olfactory sensations as there is no standard unit to quantify or measure odors as there is with other senses (e.g. hearing). There are approximately 17 000 odorous compounds (Harper, 1972) for which there is no international standardized terminology.

Individuals vary considerably in recognition of odors (Pangborn, 1981) and perception of pleasantness (Cain and Johnson, 1978). Rabin (1988) demonstrated that attaching verbal labels to olfactory stimuli significantly improved OJs ability to recognize odors. To facilitate odor assessment in this study, OJs were given a list of seven distinct odorant descriptors, which was made available during all phases of training and measurement. Although this study did not evaluate odor quality recognition, all of the study judges indicated that listing the descriptors helped them to quickly recognize/describe an odorant, which then enabled them to focus on odor intensity.

The OJs were allowed to rate how much they liked or disliked a sample. Study investigators believed that giving OJs the opportunity to rate odorant pleasantness could help to control the subjective emotional attachment a judge could have to a particular odorant, as described by Cain (1988). Stevenson *et al* (1998) have also suggested sweetness of an odorant can enhance odor intensity perception. Therefore, OJs were instructed to break down their assessment into four distinct steps, odor stimulation → rating odor pleasantness → odor description → odor intensity, similar to Shiffman's (1996) chain of sensory perception. Investigators believed these steps could enhance the learning of the intensity scale by training judges to address odor character and pleasantness before focusing on intensity. All OJs, even experienced OJs, indicated these steps helped them to focus on each aspect independently and quickly.

Cognitive and psychological factors can influence OJs perception, such as unfamiliarity with the odor or lack of training in articulating the sensation in words or numbers (Leibowitz and Post, 1980; Meilgaard *et al*, 1999b). Study investigators adapted a 10-point ASTM odorant intensity scale (n-butanol) to a 5-point intensity scale (Table 2). To familiarized OJs with the study scale, training exercises included intensity rating by comparing sample odorants to the study reference odorants n-butanol. The purpose was to eliminate OJs tendency to distribute ratings evenly over the available scale or to avoid extreme responses, the 0's and 5's (Parducci, 1965). OJs were instructed to use the n-butanol intensity reference odorants and odorant descriptors whenever they felt the need to verify or confirm their assessment. The purpose was to aid in memorizing this static scale and anchor OJs to a physical reference. Judges reported that the use of the n-butanol reference helped when a sample was very unpleasant.

An OJ can have different responses to the same stimulus on different occasions because of over stimulation of the olfactory receptors causing the phenomena of adaptation, cross-adaptation, habituation and/or fatigue (Cain and Johnson, 1978; Brinkman *et al*, 1980; Dalton and Wysocki, 1996; Rawson, 1999). A variety of distinct odorant types (floral, fruity, amines, acids, medicinal, etc.) were used to eliminate adaptation and cross-adaptation. OJs assessed samples at 3-min intervals to control fatigue. All OJs reported that the 3-min intervals seemed long that they became bored while waiting. Investigators are not sure if this loss of focus during testing had any effect on the study results.

As repeated sniffing past the first sniff rarely provides additional information (Laing, 1983), recovery time probably could be shortened.

All study OJs improved in their assessment of odor intensity from pre- to post-training for all the odorants, except for DMS for which errors became worse. Investigators mixed fresh solutions of DMS at both measurement occasions. As DMS volatizes easily, the repeated opening of the sample containers may have diluted the sample. Also sample containers were glass and sulfur-containing compounds can be reactive to glass (Sulyok *et al*, 2001).

Intensity scores differed between pleasant and unpleasant odorants when water scores were included with the pleasant odorants. However, there was no significant difference when the water score was removed from the pleasant odorant group. This suggests that offensive compounds may not be needed to train malodor judges, or that a pleasant reference odorant could be used during training.

This study was limited in that it only included six OJs. Also, only two pleasant odorants were used and there was no control group that did not receive training. True oral malodor is a mixture of compounds and this study used only pure compounds. Further studies are needed to justify the assumption that training on these odorants is transferable to other odorants. Follow-up studies should include training and assessment with mixtures of compounds and with subjects having oral malodor and identification of a reference odorant for use in studies of people with oral malodor.

Conclusions

The OJ training protocol in this pilot study improved subjects' ability to assess odor intensity irrespective of previous experience and training and is recommended for all sensory OJs prior to conducting research trials. Because study OJs did not score pleasant and unpleasant odorants differently perhaps a pleasant reference odorant could be identified and used in OJ training rather than using human subjects with oral malodor. This could reduce cost and time involved in training OJs. Few academic groups have developed training programs and as there is no standardized training protocol established and published in the oral malodor field this pilot study could possibly be used as a basis to develop a standardized training program in this field.

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ORIGINAL ARTICLE

Use of *n*-butanol as an odorant to standardize the organoleptic scale of breath odour judges

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OBJECTIVE: The alcohol *n*-butanol has been recommended for use as a standard odorant by various groups for the training or standardization of breath odour judges and sensory evaluation panels. The objective of this study is to assess the use of *n*-butanol as a suitable odorant for organoleptic training of breath judges.

METHODS: One judge with full smell acuity was trained in the method of organoleptic assessment using odorant solutions of main chemical classes (acids, amines, indole and sulphides) with the exception of alcohols. The subject was proficient in scoring odorant solutions, standard gas mixtures and human breath using the Rosenberg 0–5 organoleptic scale. A wide range of *n*-butanol solutions were prepared from 0 to 90 000 ppm and dispensed as replicate 12-ml volumes in Universal bottles (24 ml) leaving a headspace of 12 ml. Sets of odorants were prepared, labelled by code, randomized and presented to the judge in a completely blind fashion. The judge scored each concentration. This process was repeated on 32 occasions over a period of 12 weeks. Mean values of data for each determination for each concentration series were plotted against the log concentrations of odorant. Linear regression slope analysis was used to measure slope, the 95% CI of slope and the scatter of points (R^2 value). Headspace concentrations of odorant were determined using gas chromatography (GC) analysis.

RESULTS: The *n*-butanol regression slope gave a high R^2 value (0.971) and low scatter. However, the data did not correspond to other published work using an ASTM method where the range of recommended butanol concentrations was insufficient at both the high and low ends to determine the top and threshold. Moreover, headspace analysis using GC confirmed the published gas concentrations to be in error by a factor of 100. It was also observed that high concentrations of odorants were irritant causing desensitization if used for prolonged periods.

CONCLUSION: The published method had erroneous headspace calculations listed and *n*-butanol could not be recommended as a training odorant because of its irritancy.

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Keywords: *n*-butanol; organoleptic training; odour judges; organoleptic scale

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Introduction

Many compounds have been suggested as being suitable for training and calibrating odour judges. *n*-Butanol has been used previously (ASTM, 1999) as it can be obtained pure, is non-toxic, is stable in air or water and its odour is considered to be not unpleasant. It was important for us at University of the West of England as a centre for organoleptic training to determine the usefulness of *n*-butanol for our own training purposes. Therefore, the objective of our work was simply to assess the use of *n*-butanol as a suitable odorant for use in organoleptic training of breath odour judges.

Material and methods

Choice of judge

One judge with full smell acuity was trained in the method of organoleptic assessment using odorant solutions of all chemical classes (acids, amines, indole and sulphides) with the exception of alcohols. Prior to this study, the judge demonstrated her ability to distinguish odours using the smell identification tests (Sensonics Inc., Haddon Heights, NJ, USA), a method which was employed to detect specific and non-specific anosmia. The subject was proficient in scoring odorant solutions, standard gas mixtures and human breath using the Rosenberg 0–5 organoleptic scale. The judge had gained over 5 years experience of assessment and for the purposes of this work was considered to be the gold standard.

Odorant preparation and presentation
A wide range of *n*-butanol solutions were prepared from 0 to 90 000 ppm and dispensed as replicate 12-ml volumes in Universal bottles (24 ml) leaving a headspace of 12 ml. Sets of odorants were prepared, labelled by code, randomized and presented to the judge in a completely blind fashion. The judge scored each concentration. This process was repeated on 32 occasions over a period of 12 weeks.

Organoleptic scale
The modified scale of Rosenberg was employed (Greenman *et al*, 2004). The scores are described as: 0 = no odour (below the smell threshold), 1 = barely noticeable odour, 2 = slight odour, 3 = moderate odour, 4 = strong odour, and 5 = extremely strong odour close to saturation.

Gas chromatography analysis of headspace gas concentrations
Headspace concentrations of odorant were determined using gas chromatography (GC) (Hewlett Packard/HP 5890, Palo Alto, CA, USA) with automatic injection of headspace gas (1 ml). Detection was achieved using a hydrogen flame ionization detector.

Calculation of headspace gas concentration from Henry's constant
For the purposes of this work, the dimensionless Henry's law constant at 20°C at 1 atmosphere pressure for *n*-butanol was taken to be 1.1×10^2.

Expression of data
Mean values of data for each determination for each concentration series were plotted against the log concentrations of odorant. Linear regression slope analysis was used to measure slope, the 95% CI of slope and the scatter of points (R^2 value).

Results

The relationship between *n*-butanol concentrations in liquid (water) and the gas phase (12 ml + 12 ml) is shown in Figure 1. This shows both the measured values (by GC analysis) and the theoretical values calculated from Henry's constant. There was close agreement between the theoretical and measured values up to a liquid concentration of 4.0×10^{-1} (mol dm^{-3}). At higher concentrations there was a departure from the linear relationship (because of saturation of the gas phase).

The results from plotting the organoleptic scores against the log concentration of *n*-butanol (in the gas phase) are shown in Figure 2. The regression slope gave a high R^2 value (0.971) and low scatter.

The nominal concentrations for threshold (score 0) to saturation (score 5) is shown in Table 1. This table also shows figures for a recommended method given by an unnamed US-based group on ASTM (1999). There was a wide discrepancy between the two methods. The range of concentrations cited in the

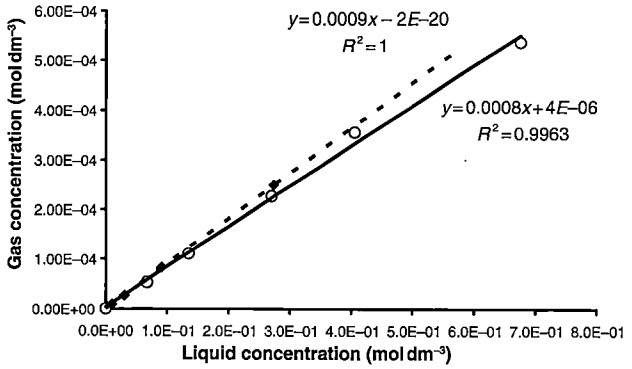

Figure 1 Gas–liquid phase relationship for *n*-butanol; correlation between liquid and gas concentration (mol dm^{-3}). Relationship determined by theory (from Henry's constant) [dotted line]. Relationship determined from GC measurements [solid line with points]

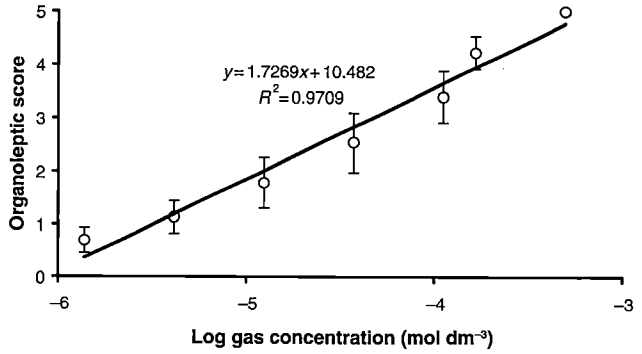

Figure 2 Relationship between organoleptic scores and log gas concentration of *n*-butanol. The error bars show ± s.d. from $n = 32$ at each concentration point

recommended method was insufficient at the high end to determine the top of the organoleptic scale and insufficient at the low end to determine threshold. Moreover, the gas concentrations cited in the recommended method were in error by a factor of over a 100-fold compared with both the theoretical values (Henry's constant) and by the practical measures using GC.

Discussion

The judge reported that high concentrations of odorants were irritant and tiring to the nose. Initial experiments conducted after exposure to the higher concentrations of *n*-butanol, suggested wider scatter in subsequent determinations. However, there are insufficient determinations to characterize this effect in quantitative terms and further work is needed. In conclusion, a previously described US method protocol had erroneous headspace calculations listed and the scale described did not reflect the organoleptic scale of the UK system. This wide discrepancy suggests the need for a more controlled approach to organoleptic training and standardization of

Table 1 Comparison of butanol liquid concentrations (ppm) and concentrations in the gas and liquid phases expressed as mol dm^{-3} with corresponding organoleptic scores for each concentration data from the University of the West of England (UWE) and a US group

Butanol liquid concentration (ppm)	*Equivalent liquid concentration (mol dm^{-3})*	*Equivalent gas concentration (ppm)*		*Equivalent gas concentration (mol dm^{-3})*		*Organoleptic scores*	
		UWE	*US*	*UWE*[a]	*US*[a]	*UWE*	*US*
0	0	0	0	0	0	0	0
10	0.001351	0.0009	–	5.51572×10^{-8}	–	0	–
250	0.033784	0.227273	25	1.37893×10^{-6}	3.380×10^{-4}	1	1
750	0.101351	0.681818	75	4.13679×10^{-6}	1.014×10^{-3}	1	2
2250	0.304054	2.045455	225	1.24104×10^{-5}	3.041×10^{-3}	2	3
6750	0.912162	6.136364	675	3.72311×10^{-5}	9.122×10^{-3}	2.5	4
20 250	2.736486	18.40909	2025	1.11693×10^{-4}	2.736×10^{-2}	3.5	5
40 500	4.054054	36.818	–	2.23386×10^{-4}	–	4	–
81 000	12.16216	73.636	–	4.46772×10^{-4}	–	5	–

[a]Gas concentrations (mol dm^{-3}) based on previously given gas ppm concentrations respectively.

organoleptic malodour judges, in order to reduce inter-judge variations, especially of those from different centres from around the world. Although the magnitude of irritancy that *n*-butanol may cause the nose has not been quantified, it could not be recommended as a training odorant because of its irritant nature.

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ORIGINAL ARTICLE

Breath odor evaluation by detection of volatile sulfur compounds – correlation with organoleptic odor ratings

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BACKGROUND: Detection of oral volatile sulfur compounds (VSC) by gas chromatography (GC) is a widely used method for evaluating breath odor. Air aspirated from the mouth is injected into the GC column for analysis. To eliminate discrepancies caused by variations in operator sampling or injection techniques, a new GC system designed to aspirate breath samples directly into the GC was developed.

OBJECTIVE: A clinical study was performed to compare this new automated breath-sampling GC system to organoleptic evaluation by two trained odor judges.

METHODS: A randomized, two-cell, double-blind, parallel design was used in which subjects were tested before and 3 h after using either a mouthrinse containing zinc or a matching placebo rinse. Thirteen subjects used the zinc mouthrinse, and 12 used the placebo. Subjects with a wide range of VSC levels were studied. The average organoleptic ratings for each subject at each time-point were compared with the average VSC measurements made with the GC, and Pearson product–moment correlation coefficients between the corresponding GC and organoleptic measurements were determined.

RESULTS: The correlation between the GC and organoleptic assessment methods were highly significant ($P \leq 0.001$) for: total VSC, 0.65; H_2S, 0.63; CH_3SH, 0.61; and $(CH_3)_2S$, 0.46. The correlation between the two judges was also highly significant (0.823, $P < 0.001$).

CONCLUSION: These results demonstrate the utility of the automated GC method for evaluating breath odor.

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Keywords: volatile sulfur compounds; gas chromatography; halitosis; oral malodor; breath

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Introduction

Detection of volatile sulfur compounds (VSC) by gas chromatography (GC) is a widely used method for evaluating breath odor (Tonzetich, 1971; Solis-Gaffar *et al*, 1975). Air aspirated from the mouth is directly injected into the GC column for analysis. To eliminate discrepancies caused by variations in operator sampling or injection techniques, a new GC system designed to aspirate breath samples directly into the GC was developed. This method allows individual quantification of the three main VSC's in mouth air, hydrogen sulfide, methyl mercaptan, and dimethyl sulfide. A clinical study was performed to compare this new automated breath-sampling GC system to organoleptic evaluation by two trained odor judges.

Methods

A randomized, two-cell, double-blind, parallel design was used in which subjects were tested before and 3 h after using either a prototype mouthrinse containing 0.4% zinc acetate or a matching placebo rinse without zinc. During the 7-day period prior to the study, all subjects used a regular fluoride dentifrice (Colgate-Palmolive Company, New York, NY, USA). Thirteen subjects rinsed with 15 ml of the zinc mouthrinse, and 12 with 15 ml of the placebo for 60 s. Subjects with a wide range of VSC levels were studied. On the morning of the study, subjects arrived at the clinical facility, without eating, drinking, or performing oral hygiene, for baseline evaluations. After a limited breakfast, subjects rinsed with their assigned product for 1 min and returned 3 h later for post-treatment evaluations.

Instrumental analysis of breath air was performed using a GC system consisting of an Agilent 6890 gas chromatograph (Wasson-ECE Instrumentation, Ft Collins, CO, USA) with a flame photometric detector and an automated system that aspirates gaseous samples from the mouth and injects them onto the column. The chromatography method used was able to quantify the three individual volatile sulfur species hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, at levels

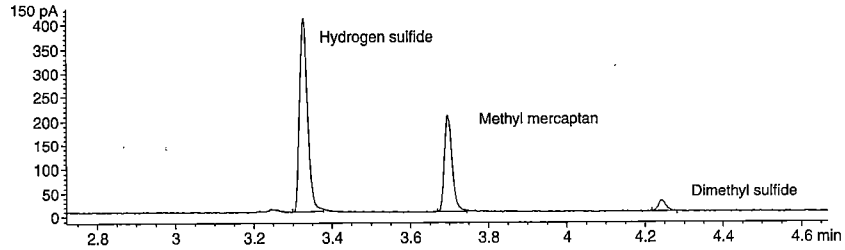

Figure 1 Sample chromatogram of mouth air

Table 1 Organoleptic rating scale

Mouth odor rating	*Malodor*
0	None
1	Barely
2	Slight
3	Moderate
4	Strong
5	Very strong

commonly found in the mouth (Tonzetich, 1971, 1977). For each sample, concentrations of these three species were calculated and added to determine the total VSC concentration. A sample chromatogram is shown in Figure 1.

Sensory breath evaluation was performed by two trained, calibrated breath examiners. Mouth air samples were collected from each subject in triplicate using a specially designed mouthpiece and 20-ml polypropylene syringes. Following mouth air sampling, the syringes were blindly and randomly presented to each odor judge for assessment using a scale of 0–5 (Table 1). The scores given each sample by the two judges were averaged to determine an overall organoleptic rating for that sample. The average organoleptic ratings for each subject at each time-point were compared with the average VSC measurements made with the GC, and Pearson product–moment correlation coefficients between the corresponding GC and organoleptic measurements were determined.

Individual product effects were determined by comparing the initial pretreatment ratings to the 3-h ratings for each product tested using a paired *t*-test. The effects of the two products were compared using an ANCOVA performed on the 3-h measurements with the products and the baseline measurements as covariants.

Results

Table 2 lists the correlation coefficients between the individual and total VSC measurements and the organoleptic ratings. The significance level (*P*-value) of each correlation is also listed. The correlations between the organoleptic ratings and both total VSC and the individual sulfur species were all statistically significant ($P < 0.001$), with the total VSC having the highest correlation to the mouth odor ratings. The correlation between the two judges was also highly significant ($P < 0.001$) with a correlation coefficient of 0.823, indicating a strong degree of agreement between the perceptions of mouth odor by the two judges.

Table 2 Correlation coefficients between GC measurements and organoleptic ratings

GC measurement	R^a	*Statistical significance*
Total VSC	0.6481	<0.001
H_2S	0.6336	<0.001
CH_3SH	0.6120	<0.001
$(CH_3)_2S$	0.4642	<0.001

[a]Pearson product–moment correlation coefficients (*R*) and significance levels for comparisons between specific GC measurements and organoleptic ratings.
GC, gas chromatography; VSC, volatile sulfur compounds.

Table 3 Baseline and 3-h levels for each treatment group (mean ± s.d.)

	0.4% zinc acetate rinse		*Placebo rinse*	
	Baseline	*3-h*	*Baseline*	*3-h*
Total VSC (p.p.b.)	532 ± 346	35 ± 68	574 ± 468	397 ± 378
Organoleptic ratings	3.6 ± 1.0	1.2 ± 0.8	3.1 ± 1.0	2.3 ± 1.1

Mean organoleptic ratings and mean total VSC at each time-point are shown with standard deviations in Table 3. Compared with baseline, both the GC method and the organoleptic method demonstrated a statistically significant 3-h effect for the zinc-containing mouth rinse ($P < 0.05$) and no significant effect for the placebo ($P > 0.05$). Both methods also demonstrated a statistically significantly greater effect for the active rinse compared with the placebo ($P < 0.05$).

Discussion and conclusion

The results of this study, demonstrate that there is a statistically significant correlation between VSC in mouth air as measured by the breath-sampling GC system and organoleptic evaluations of mouth odor. Compared with the results presented by Schmidt *et al* (1978), the VSC measurements observed here indicate comparable or stronger correlations with the organoleptic ratings. The two methods also performed similarly in evaluating the effects of the two mouthrinses tested. Features of the current system that contribute to this performance include the automated breath-sampling assembly, a chromatographic method specially designed

to separate and detect VSCs found in the oral cavity, and computerized data acquisition and analysis methods. These results demonstrate the utility of the automated GC method for evaluating breath odor.

Acknowledgment

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ORIGINAL ARTICLE

Clinical effectiveness of a triclosan/copolymer/sodium fluoride dentifrice in controlling oral malodor: a 3-week clinical trial

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OBJECTIVE: The purpose of the study was to compare the effectiveness of a dentifrice containing 0.3% triclosan, 2% polyvinylmethylether/maleic acid (PVM/MA) copolymer, 0.243% sodium fluoride (TCF) to a commercially-available dentifrice containing 0.243% sodium fluoride (control) for the management of oral malodor in a 3-week, randomized double-blind, longitudinal clinical trial.

METHODS: A panel of four expert judges used a nine-point hedonic scale to evaluate breath odor using a protocol designed in accordance with the ADA Draft Acceptance Program Guideline for Products Used in the Management of Oral Malodor. Following a baseline evaluation, prospective subjects with hedonic scores above the threshold value for unpleasant breath were stratified by score and randomized into two treatment groups. Subjects brushed their teeth for 1 min with their assigned dentifrice after which they were scored for oral malodor at 1.5, 4 and 12 h. They then used their assigned dentifrice, twice a day, for 3 weeks. Before oral malodor evaluations, the subjects refrained from eating odorigenic foods, using mouthrinses and breath mints and from performing dental hygiene procedures.

RESULTS: Eighty-one adult male and female subjects completed the study. The baseline hedonic scores for the TCF and control dentifrices were 7.80 and 7.84, respectively, corresponding to unpleasant breath. The final mean oral malodor scores for the TCF dentifrice differed significantly from the baseline and control values ($P < 0.05$) for every time point examined (1.5-, 4-, 12-h and 1-, 2- and 3-week intervals). The mean final breath scores for the TCF dentifrice group were 3.06, 3.48, 3.42, 3.66, 3.41 and 3.36, respectively, for each time point. These scores correspond to pleasant breath. Conversely, the control dentifrice group scored at levels either above 5.0 (before 12 h) or above 7.0 (after 12 h) which corresponded to neutral or unpleasant breath.

CONCLUSION: In conclusion, the results of this double-blind clinical study clearly indicate that a dentifrice containing triclosan/copolymer/NaF provides effective control of oral malodor for up to 12 h.
Oral Diseases (2005) 11 (Suppl. 1), 51–53

Keywords: halitosis; triclosan; oral malodor; dentifrice; breath

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Introduction

The objective of this study was to compare the effectiveness of a test dentifrice containing triclosan/copolymer/sodium fluoride to a commercially-available, clinically-proven, ADA-accepted, sodium fluoride control dentifrice for the management of oral malodor in a 3-week, randomized, double-blind, longitudinal clinical trial.

Materials and methods

Products tested

Two commercially-available toothpastes, one containing 0.3% triclosan, 2% copolymer and 0.243% sodium fluoride in a silica base (Colgate-Palmolive Company, New York, USA) and the other containing 0.243% sodium fluoride in a silica base (Colgate-Palmolive Company) were evaluated.

Protocol design

The study employed a double-blind, stratified, two-treatment design, and followed a protocol set forth in the proposed ADA Guidelines for Acceptance of products used for the management of oral malodor (ADA, 2003). Eighty-one adult male and female subjects between 18 and 70 years of age and in good oral and general health took part in the 3-week clinical trial (Table 1). Subjects refrained from all oral hygiene and from eating, drinking and smoking on the morning of the baseline oral malodor examination. The olfactory acuity of a trained four member judge panel was verified

Table 1 Summary of age and sex characteristics of the study participants

	Number of subjects			*Age (years)*	
Dentifrice group	*Male*	*Female*	*Total*	*Mean*	*Range*
TCF-AF	22	19	41	45.12	22–70
Control	21	19	40	44.33	26–68

prior to each evaluation. Oral odor rating was done using a nine-point hedonic scale (1 = most pleasant, 5 = neutral and 9 = most unpleasant). Subjects who presented scores above a threshold value for unpleasant breath odor were stratified by score and randomized into two treatment groups, using the test or control dentifrice. They were instructed to brush their teeth thoroughly in their regular and customary manner for 1 min with their assigned dentifrice.

Odor evaluation

Hedonic scoring of oral malodor was done by four independent, experienced, calibrated judges at 1.5, 4 and 12 h after the first 1-min product use. Subjects were then instructed to brush their teeth twice per day over the next 3 weeks. They returned to the clinical facility on days 8, 15 and 22 for morning appointments, approximately 12 h after their last tooth brushing, and having refrained from eating and drinking, and did not use dental hygiene procedures, breath mints, or mouthrinses. Subjects received the same hedonic oral malodor examination as described above. Following individual scoring, an overall score was determined for each subject by averaging the scores assigned by the four judges.

Statistical analysis

The within-treatment comparisons between the baseline and each time point (1.5 h, 4 h, 12 h, 1 week, 2 weeks and 3 weeks) scores were performed using paired *t*-tests. Comparisons between the study dentifrices with respect to oral malodor scores at baseline and at each test time point after tooth brushing were performed using an analysis of covariance, in which baseline oral malodor was employed as a co-variable. All statistical tests of hypotheses were two-sided, and employed a level of significance of $\alpha = 0.05$.

Results and discussion

There was no statistically significant difference between the mean oral malodor scores for both groups at baseline. The range of values corresponded to *unpleasant* breath. At all measured time points, a statistically significant difference was observed between the triclosan/copolymer/sodium fluoride dentifrice and control dentifrice groups (Tables 2 and 3). The mean malodor scores were within the range corresponding to pleasant breath for the test dentifrice and for the control dentifrice, the values were within the neutral to offensive range (Figures 1 and 2). The percentage of subjects having a reduction of at least a 3.2 unit of their baseline

Table 2 Summary of the daytime breath-odor scores evaluated on day 1 for subjects who completed the clinical study

Dentifrice group	*n*	*Baseline*	*1.5 h*	*4 h*	*12 h*
TCF-AF	41	7.80 ± 0.42	3.06 ± 0.67*	3.48 ± 0.82*	3.42 ± 0.72*
Control	40	7.84 ± 0.39†	5.36 ± 0.65	5.84 ± 0.67	7.03 ± 0.70

*TCF-AF was significantly better than control ($P \leq 0.05$).
†No significant difference between baseline values ($P \geq 0.05$).

Table 3 Summary of the 12-h overnight breath-odor scores at the weekly follow-up evaluation for subjects who completed the clinical study

Dentifrice group	*n*	*Baseline*	*1 Week*	*2 Weeks*	*3 Weeks*
TCF-AF	41	7.80 ± 0.42	3.66 ± 0.50*	3.41 ± 0.53*	3.36 ± 0.50*
Control	40	7.84 ± 0.39†	7.14 ± 0.81	7.16 ± 0.43	7.12 ± 0.34

*TCF-AF was significantly better than control ($P \leq 0.05$).
†No significant difference between baseline values ($P \geq 0.05$).

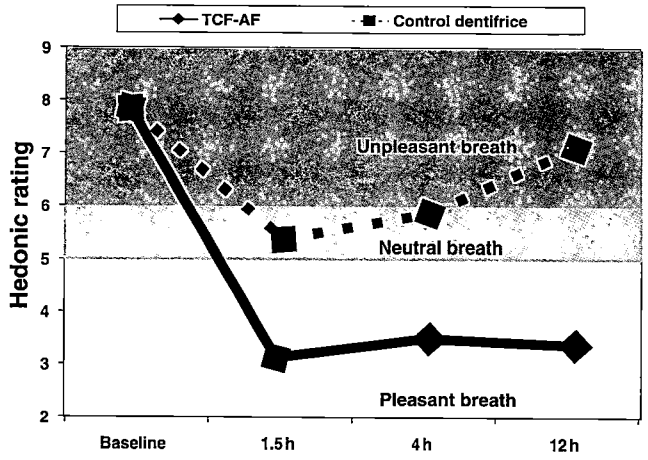

Figure 1 Twelve-hour daytime hedonic scores at day 1 evaluations

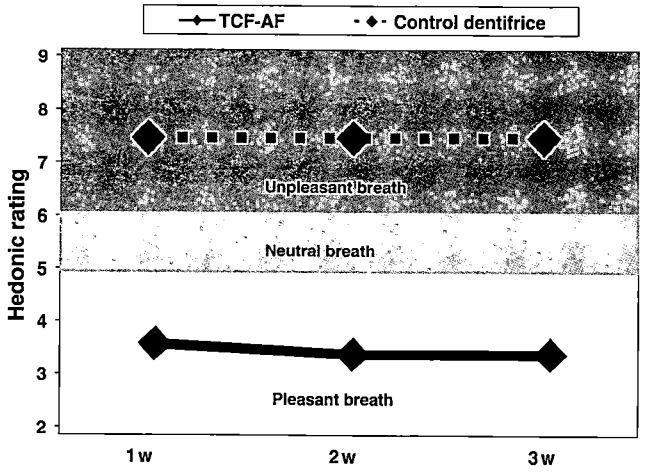

Figure 2 Twelve-hour overnight scores after 3 weeks product use

Table 4 Percentage of subjects exhibiting reduction ≥3.2 units relative to baseline

Dentifrice group	*1.5 h*	*4 h*	*12 h*	*1 Week*	*2 Weeks*	*3 Weeks*
TCF-AF	97.6	87.8	92.7	92.7	95.1	97.6
Control	10.0	10.0	0.0	2.5	0.0	0.0

breath odor scores ranged from 87.8 to 97.6% for the TCF dentifrice and from 0.0 to 10% for the control dentifrice at the measured time points (Table 4).

The copolymer in the triclosan/copolymer/sodium fluoride formula has been shown to enhance the delivery and retention of the antibacterial agent triclosan to oral surfaces (Nabi *et al*, 1989; Gaffar *et al*, 1990, 1994). A study by Kruger *et al* (1996) demonstrated that the concentration of triclosan in plaque biofilm 12 h after brushing the teeth was sufficient to inhibit the growth of bacteria, therefore, retard the return of bad breath. Additionally, the formula was demonstrated to control oral malodor as assessed by a panel of hedonic judges (Sharma *et al*, 1999). The study results indicate that a dentifrice containing 0.3% triclosan/2% PVM/MA copolymer/0.243% sodium fluoride, commercially sold as Colgate® Total® Advanced Fresh™ Gel, is effective in controlling daytime and overnight bad breath for up to 12 h, and that its clinical end points satisfy the proposed ADA Draft Acceptance Program Guidelines for Products Used in the Management of Oral Malodor.

In conclusion, the results of this double-blind clinical study clearly indicate that a dentifrice containing triclosan/copolymer/sodium fluoride provides effective control of oral malodor for up to 12 h.

Acknowledgment

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ORIGINAL ARTICLE

The clinical comparison of a triclosan/copolymer/fluoride dentifrice *vs* a breath-freshening dentifrice in reducing breath odor overnight: a crossover study

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OBJECTIVE: The objective of this randomized, crossover study was to compare the effectiveness of the triclosan/copolymer/sodium fluoride (TCF-AF) dentifrice and a commercially available breath-freshening dentifrice containing fluoride for their ability to reduce volatile sulfur compounds (VSC) associated with oral malodor overnight.

METHODS: Following a 1-week washout period of brushing with a regular fluoride dentifrice, subjects reported to the clinical site without performing oral hygiene, eating or drinking in preparation for baseline breath sampling. Subjects were randomly assigned a test dentifrice and instructed to brush their teeth for 1 min, twice a day for 1 week. On the morning of day 8, subjects returned to the test site, having refrained from oral hygiene, eating and drinking, for overnight sampling. Following a second 1-week washout period, subjects repeated the same regimen, but now using the other test product. At each measurement, the level of breath VSC was evaluated using a gas chromatograph equipped with a flame photometric detector. Measurements were taken in duplicate, and then averaged. The levels of VSC were expressed as parts per billion (ppb) in mouth air.

RESULTS: At baseline, the mean breath VSC levels for the TCF-AF and breath-freshening dentifrice were 618 and 581 ppb respectively. There was no statistically significant difference between the baseline levels. Overnight, the TCF-AF and the breath-freshening dentifrice reduced breath mean VSC levels to 267 and 521 ppb respectively. This gave a 56.7 and 10.2% reduction in VSC levels for these two products, respectively, compared with baseline. The reduction for the TCF-AF dentifrice was significantly different ($P < 0.05$%) from that of the breath-freshening dentifrice.

CONCLUSION: The results of this randomized, double-blind, crossover study indicate that the TCF-AF dentifrice was significantly more effective than a commercially available breath-freshening dentifrice containing fluoride in reducing breath VSC associated with bad breath overnight.

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Keywords: triclosan/copolymer dentifrice; fluoride; fresh breath; gas chromatography; oral malodor; volatile sulfur compounds

Introduction

The objective of this randomized, crossover study was to compare the effectiveness of the triclosan/copolymer/sodium fluoride (TCF-AF) dentifrice and a breath-freshening dentifrice containing fluoride for their ability to reduce volatile sulfur compounds (VSC) associated with oral malodor overnight.

Methods

Products tested

Two commercially available toothpastes, one containing 0.3% triclosan, 2% copolymer and 0.243% sodium fluoride in a silica base (Colgate-Palmolive Company, New York, NY, USA) and the other containing 0.243% sodium fluoride in a silica base (Proctor & Gamble Company, Cincinnati, OH, USA), were evaluated.

Clinical procedure

Healthy adult male and female subjects, age 21–60 years with a VSC level >300 ppb who signed the informed consent form were entered into the study. The design was a randomized, multiple-use, crossover study.

On day 1 of the study, subjects reported to the clinical facility without eating, drinking or performing oral hygiene for baseline breath VSC evaluation. There, each subject's breath odor was evaluated using a Wasson–ECE custom-built breath sampling gas chromatography system equipped with a flame photometric detector (Agilent

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Table 1 Summary of age and sex characteristics of the study participants

Product	No. of subjects			Age range
	Male	Female	Total	
TCF-AF	12	5	17	21–60
Commercially available breath-freshening dentifrice	12	5	17	21–60

Model # 6890 Gas Chromatograph Agilent Technologies, Palo Alto, CA, USA). This method was previously described by Tonzetich (1971) and later by Niles and Gaffar (1995). Subjects were provided with a soft-bristled adult toothbrush, and instructed to brush their teeth with the assigned dentifrice twice a day for 1 min. They used the product for a period of 7 days. On the evening of day 7, subjects were instructed to brush their teeth just prior to retiring to bed for the night. The following morning (day 8), subjects reported to the clinical site without eating, drinking or performing oral hygiene for their overnight evaluation. During a 7-day washout period, subjects used a basic fluoride dentifrice and repeated the same regimen now using the other dentifrice.

Upon arrival at the testing facility, subjects were instructed to keep their mouths closed for 10 min. A sampling tube was slid between the lips and back of the teeth. With the mouth closed, gas was pulled from the subject's mouth directly into the inlet of the gas chromatograph through the gas sampling loop, which was used to inject 2 ml of the sample into the column for analysis. Subjects were instructed to perform the Val-Salva maneuver during sampling to ensure air was removed only from the mouth and not through the nasal passages or from the lungs. The entire sampling and measurement process was performed in duplicate at each evaluation, and the resulting two scores were averaged. The duplicate samples were analyzed for three VSC gases (hydrogen sulfide, methyl mercaptan and dimethyl sulfide) commonly found in mouth air.

Statistical method

For both products, paired *t*-tests were performed to compare overnight VSC levels to baseline VSC levels. Analysis of covariance was performed on the overnight results, using the baseline as the covariate. Treatments were declared statistically and significantly different if $P \leq 0.05$.

Results

A summary of the age and sex characteristics of the subjects who participated in the study is presented in Table 1. The TCF-AF dentifrice reduced breath VSC from 618 to 267 ppb, representing a 57% reduction compared with baseline. However, the commercially available breath-freshening dentifrice reduced breath VSC from 581 to 521 ppb, representing a 10.1% reduction compared with baseline (Table 2). The TCF-AF dentifrice was statistically significantly ($P < 0.05$) better than the commercially available breath-freshening dentifrice in reducing breath VSC associated with bad breath overnight (Table 3).

Table 2 Mean overnight post-treatment VSC scores after brushing with Colgate® Total® Advanced Fresh™ Dentifrice and a commercially available fluoride dentifrice

Product	N	Mean baseline VSC levels (ppb)	Mean overnight VSC levels (ppb)	% Reduction compared with baseline
TCF-AF	17	618.1 ± 269.9	267.4 ± 139.8	57
Commercially available breath-freshening dentifrice	17	580.9 ± 264.6	521.4 ± 217.3	10

Table 3 Significance of reduction compared with the commercial breath-freshening dentifrice

Product	Reduction of VSC levels from baseline (ppb)	Significance[a]
TCF-AF	330.7	$P < 0.05$
Commercially available breath-freshening dentifrice	59.5	

[a]When the overnight breath VSC scores were compared, the TCF-AF dentifrice produced statistically significantly less breath VSC than the breath-freshening dentifrice.

Discussion

The copolymer in the TCF-AF formula has been shown to enhance the delivery and retention of the antibacterial agent triclosan to oral surfaces (Nabi *et al*, 1989; Gaffar *et al*, 1990, 1994). Additionally, the formula was demonstrated to control oral malodor as assessed by a panel of hedonic judges (Sharma *et al*, 1999; Hu *et al*, 2003). This double-blind crossover clinical study demonstrated

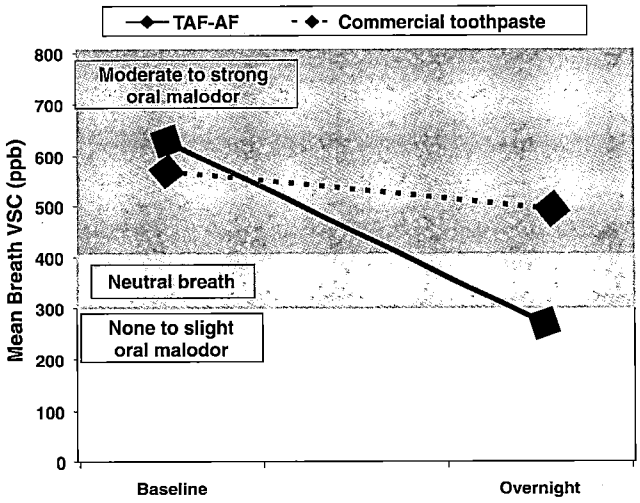

Figure 1 Comparison of overnight breath VSC after brushing the teeth

that a new variant of TCF-AF dentifrice, sold commercially as Colgate® Total® Advanced Fresh™ Gel, significantly reduced VSC associated with oral malodor overnight compared with baseline as well as a commercially available breath-freshening dentifrice. It was shown by Hunter *et al* (2003) that breath VSC levels below 300 ppb corresponded to subjects having no or slight oral malodor, while a breath VSC level above 400 ppb corresponded to subjects having moderate to strong oral malodor. The final mean overnight breath VSC level for the TCF-AF group was in the range corresponding to no or slight breath odor whereas, the final mean overnight breath VSC for the commercially available dentifrice was in the range corresponding to moderate to strong oral malodor (Figure 1).

The results of this randomized, double-blind, crossover study indicate that the TCF-AF dentifrice was significantly more effective than a commercially available breath-freshening dentifrice containing fluoride in reducing breath VSC responsible for bad breath overnight.

Acknowledgments

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ORIGINAL ARTICLE

Effect of a triclosan/PVM/MA copolymer/fluoride dentifrice on volatile sulfur compounds *in vitro*

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OBJECTIVE: The objective of the investigation was to document the *in vitro* efficacy of a triclosan/PVM/MA copolymer/fluoride (TCF) dentifrice against the formation of volatile sulfur compounds (VSC) as well as the growth of H_2S-producing bacteria. Clinical studies using organoleptic judges, gas chromatography, or a portable sulfide monitor have generally been employed in the assessment of treatments for the control of oral malodor. However, these studies are not appropriate for screening purposes because of the expense and time required.

METHODS: An *in vitro* method was developed for the purpose of screening new compounds, agents or formulations for their ability to control VSC formation and for determining bio-equivalence of efficacy when implementing changes in existing formulations. The method combines basic microbiological methods, dynamic flow cell techniques and head space analysis. The *in vitro* VSC method was validated by comparing the efficacy of two dentifrices containing TCF with a control fluoride dentifrice as the TCF products have been clinically proven to control oral malodor.

RESULTS: In the validation studies, the TCF-containing dentifrices were significantly better ($P < 0.05$) than the control dentifrice in inhibiting VSC formation and reducing H_2S-producing bacteria. For example, when compared with baseline, the TCF dentifrices reduced VSC formation between 42 and 49% compared with the control dentifrice which reduced VSC formation 3%. There was no significant difference ($P > 0.05$) between the two TCF dentifrice formulations.

CONCLUSION: Using an *in vitro* breath VSC model, it has been demonstrated that two variants of a dentifrice containing triclosan, PVM/MA copolymer and fluoride have efficacy that is significantly better than a control fluoridated dentifrice and that there is no significant difference between the triclosan/PVM/MA copolymer/fluoride dentifrice variants.

Oral Diseases (2005) 11 (Suppl. 1), 57–60

Keywords: copolymer; flow cell system; oral malodor; triclosan; dentifrice; saliva; *in vitro* method

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Introduction

The objective of the investigation was to document the *in vitro* efficacy of a triclosan/PVM/MA copolymer/fluoride (TCF) dentifrice against the formation of volatile sulfur compounds (VSC) as well as the growth of H_2S-producing bacteria. Clinical studies using organoleptic judges, gas chromatography, or a portable sulfide monitor have generally been employed in the assessment of treatments for the control of oral malodor. However, these studies are not appropriate for screening purposes because of the expense and time required.

Materials and methods

Products tested

Two triclosan/copolymer/sodium fluoride variants, the original Colgate® Total® formula (TCF-O) and a new formula with a high impact flavor, Colgate® Total® Advanced Fresh® (TCF-AF), were compared with a control dentifrice, Colgate® Anti-cavity dentifrice (Colgate-Palmolive Company, Piscataway, NJ, USA). Slurries of the dentifrices were prepared by mixing dentifrice and water in a 1:2 (w/w) ratio.

Growth inhibition

Saliva was collected from healthy human subjects and clarified by centrifuging for 20 min at 7840 *g*. Hydroxyapatite (HAP) disks were coated with clarified saliva for 30 min then incubated with 5 ml of dentifrice slurry in a 37°C water bath for 30 min. Subsequently, each disk, after rinsing twice with deionized sterile water was placed in 5 ml of trypticase soy broth inoculated with *c.* 10^6 cells of *Actinomyces naeslundii* (ATCC no. 43146). The optical density at 610 nm of the bacteria mixture was read after incubating for 0, 4 and 24 h at 37°C. The percentage reduction in bacterial growth was calculated *vs* the water control.

Zone of inhibition

Saliva-coated HAP disks were treated with dentifrice slurries and rinsed with water as described above. Bacteria from the back of the tongue were collected from the same healthy individual at time of use and

plated onto 'oral H_2S organisms' agar (OHO-C) containing lead acetate (Anaerobe Systems, Morgan Hill, CA, USA) to detect oral bacteria producing hydrogen sulfide (El-Halabi *et al*, 1999; Paryavi-Gholami *et al*, 1999). Each dentifrice slurry treated-HAP disk was placed in the center of the lawned agar plate, which was then incubated anaerobically at 37°C for 48 h. The distance from the edge of the clear area to the edge of the disk (zone of inhibition) was measured. The edge of the clear area was defined as the junctions between the clear zone and the growth area of the dark pigmented colonies. Products were tested in triplicate.

In vitro *VSC assessment*

The flow cell system, previously described by Pilch *et al* (2004), was modified by adding an air-tight headspace vial to collect gases formed from bacterial putrefaction (Figure 1). There were four sample chambers per flow cell with each having a total volume of about 5 ml. The system was housed in a 37°C incubator. Saliva from healthy individuals, diluted twofold with water and supplemented with 1% fluid thioglycollate broth (Becton, Dickinson, and Company, Sparks, MD, USA) served as the bacterial seed. To evaluate the effect of the test dentifrices on VSC formation, saliva-coated HAP disks were treated with dentifrice slurries as described above and then transferred to the sample chambers of the flow cell. The disks were then rinsed by flowing artificial saliva at a flow rate of 1 ml min^{-1} for 1 h (single pass through) to remove loosely bound materials. Supplemented saliva was then circulated through the flow cell system overnight at a steady flow rate of 0.1 ml min^{-1}. The generated VSC was trapped in the headspace vial and analyzed using an Agilent 6890 gas chromatography equipped with a flame photometric detector (Agilent Technologies, Palo Alto, CA, USA). An untreated cell was included in each study and used as a negative control. Efficacy was assessed by semi-quantitative means using the change (reduction) in the sum of the observed response values of the three volatile sulfur gases (hydrogen sulfide, methyl mercaptan, and dimethyl sulfide) for the test dentifrices compared with the negative control.

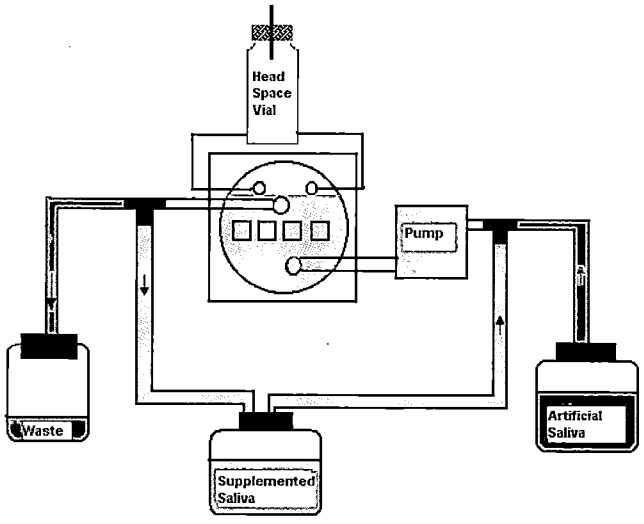

Figure 1 Schematic representation of the individual flow cell system. Gases produced by bacterial putrefaction are trapped in the tightly-sealed headspace vial

Post-reaction mixture assessment

The resulting supplemented saliva mixture from the *in vitro* VSC experiment was serially diluted with phosphate-buffered saline solution. The 10^{-3} and 10^{-4} dilutions were plated in duplicate onto OHO-C plates and incubated for 48 h at 37°C. The dark pigmented colonies were counted and expressed as log CFU.

Results

Antimicrobial efficacy of Colgate® Total® Advanced Fresh

Growth inhibition: The ability of the tested dentifrices to affect bacterial growth was evaluated using *A. naeslundii*, an early colonizer in dental plaque. Although there is no reported link between *A. naeslundii* and oral malodor formation, similar bacteria reduction profile has been observed between *A. naeslundii* and *F. nucleatum*, a known oral malodor former (data not shown), when treated with triclosan-containing products. The TCF-AF and the TAF-O formula reduced bacterial growth 59 and 51%, respectively, compared with water; whereas, the control dentifrice provided a 10% reduction in bacterial growth compared with water (Table 1). There was no significant difference ($P > 0.05$) between the two TCF variants. However, both were significantly ($P < 0.05$) better than the control dentifrice.

Zone of inhibition: The antimicrobial activity of the two triclosan/copolymer/sodium fluoride dentifrice variants was also observed on OHO-C plates using an *in vitro* diffusion method. The negative control dentifrice produced a zone that was 3.1 mm in diameter (Table 2). Both TCF variants produced similar zone of inhibition, 8.2 mm for TCF-O and 8.4 mm for TCF-AF (Table 2). There was no significant difference ($P > 0.05$) between the two variants, although both were significantly ($P < 0.05$) better than the control dentifrice.

Table 1 Percent bacterial growth inhibition after treatment with two variants of the triclosan/copolymer/ fluoride dentifrice and a control dentifrice

Product	*% Growth inhibition*[a]	*Statistical significance P*-value[b]
TCF dentifrice	51	<0.05
TCF-AF dentifrice	59	<0.05
Control dentifrice	5	>0.05

[a]Within-treatment percent reduction between untreated cell values and post-treatment values, expressed as a percentage of the untreated cell values.
[b]Significance of within treatment paired *t*-test comparison of the untreated cell values *vs* post-treatment values.

Table 2 Zone of tongue bacterial growth inhibition after treatment with two variants of the triclosan/copolymer/fluoride dentifrice and a control dentifrice (mean ± s.d.)

Product	*Zone of inhibition (mm)*
TCF	8.2 ± 1.7
TCF-AF	8.4 ± 1.2
Control	3.1 ± 1.5

VSC formation in vitro

A modification of the *in vitro* model, first reported by Pilch *et al* (2004), was employed in the evaluation of two triclosan/copolymer/sodium fluoride dentifrice variants for the ability to reduce the formation of VSC associated with oral malodor. The TCF-AF formula displayed a similar percentage reduction in VSC formation compared with the TCF-O formula. The respective reduction in VSC formation for the TCF-AF and TCF-O dentifrices was 49 and 42% compared with the negative control (Figure 2). Both were significantly better ($P < 0.05$) than the control dentifrice which reduced VSC by approximately 3%.

Post-reaction mixture

The resulting bacterial mixture was analyzed for H_2S-producing bacteria using OHO-C agar which reveal H_2S-producing bacteria as dark pigmented colonies. The results, shown in Figure 3, indicated that the TCF-AF dentifrice provided bacterial reduction of 36% compared with the negative control, which was similar to the TCF-O formula (33%). Both variants were significantly better in reducing H_2S-producing bacteria than the control dentifrice.

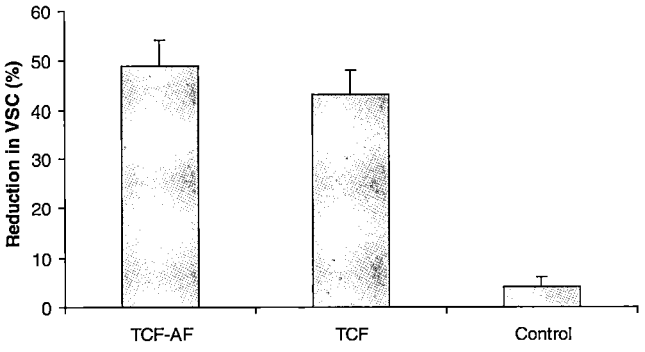

Figure 2 Percent reduction of VSC after treatment with two variants of the triclosan/copolymer/fluoride dentifrice and a control dentifrice *in vitro*

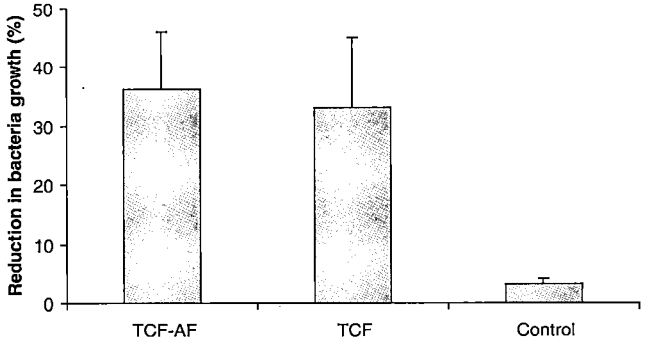

Figure 3 Percent reduction of H_2S-producing bacteria in the postreaction mixture after treatment with two variants of the triclosan/copolymer/fluoride dentifrice and a control dentifrice *in vitro*

Discussion

Three *in vitro* models were used to evaluate the efficacy of a new triclosan/copolymer/sodium fluoride dentifrice variant compared with the original formula and a control toothpaste. The triclosan/copolymer/sodium fluoride technology has been shown to reduce the common dental infections, caries and periodontal disease, as well as control common oral conditions such as tartar and oral malodor (Sharma *et al*, 1999; Mann *et al*, 2001; Allen *et al*, 2002; Mankodi *et al*, 2002). The TCF-AF dentifrice was shown to provide similar antibacterial activity to the original TCF formula and both were significantly better that a control dentifrice.

Many *in vitro* models developed to evaluate the effect of treatment for the control of oral malodor employ static conditions (Richter and Tonzetich, 1964; Solis-Gaffar *et al*, 1979; Kleinberg and Codipally, 1999) or in the case of dynamic models, concurrent entrapment of generated volatile sulfur gases is not achieved (Pratten *et al*, 2003). An *in vitro* breath VSC model, which incorporates flow dynamics and concurrent entrapment of volatile sulfur gases, was used previously (Pilch *et al*, 2004) to demonstrate the efficacy of a new triclosan/copolymer/fluoride variant (TCF-O) in reducing the formation of VSC. In that study (Pilch *et al*, 2004), the TCF-O dentifrice was shown to reduce VSC formation on average 35% *vs* a control dentifrice in a series of five experiments. It was expected that the TCF-AF dentifrice would behave similarly and the results of this study indicate that there was no significant difference between the TCF-AF and TCF-O variants in reducing VSC and H_2S-producing bacteria. Both variants were significantly more effective than a control dentifrice in reducing the measured factors. In a clinical study, Williams and Niles (2003) have demonstrated there was no significant difference between TCF-AF and TCF-O in their ability to control breath VSC measured by GC. Additionally, the results observed using the *in vitro* model were also supported by findings of Niles *et al* (1999) which showed that the triclosan and copolymer technology provided long-lasting control of breath VSC compared with a control dentifrice. These results are further supported by two *in vitro* microbiological methods which showed that the two TCF variants provided sustained antibacterial activity against oral bacteria. Together, these findings indicate that the *in vitro* breath VSC model may be a useful screener of developing formulations for the treatment of oral malodor before initiating expensive clinical trials.

In conclusion, a predictive *in vitro* breath VSC model, which incorporated flow dynamics, has been developed and used to demonstrate that two variants

of a dentifrice containing triclosan, PVM/MA copolymer and sodium fluoride have efficacy that is significantly better than a control fluoridated dentifrice and that there is no significant difference between the triclosan/PVM/MA copolymer/fluoride dentifrice variants.

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ORIGINAL ARTICLE

Microbiological culture analysis of the tongue anaerobic microflora in subjects with and without halitosis

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OBJECTIVE: Determination of the microflora present on the tongue dorsum of subjects with and without halitosis using conventional microbiological culture methods.
METHODS: Twenty-one halitosis and 20 control patients were recruited using a strict clinical protocol. Samples were collected from the posterior dorsum of the tongue using a sterile brush. Each sample was vortex mixed for 30 s and serial 10-fold dilutions to 10^{-7} were carried out. Samples were plated onto fastidious anaerobe agar (FAA) and FAA enriched with vancomycin. These were incubated under anaerobic conditions for 10 days at 37°C. Strict anaerobes were identified by metronidazole sensitivity and bacteria were identified to genus level by a combination of colony morphology, Gram staining and biochemical and enzymatic tests (rapid ID 32 A).
RESULTS: The predominant species in test and control groups were *Veillonella* sp. and *Prevotella* sp. Greater species diversity was found in the halitosis samples compared with controls. The halitosis samples contained an increased incidence of unidentifiable Gram-negative rods, Gram-positive rods and Gram-negative coccobacilli.
CONCLUSIONS: There was no obvious association between halitosis and any specific bacterial genus. The increased species diversity found in halitosis samples suggests that halitosis may be the result of complex interactions between several bacterial species. The role of uncultivable bacteria may also be important in contributing to this process.
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Keywords: halitosis; bacteria; microbiological culture

Introduction

Few studies have sought to examine and identify the tongue microflora from healthy subjects or from those with halitosis. The role of bacteria in producing oral malodour by salivary protein degradation is well established. McNamara *et al* (1972) correlated the production of malodour *in vitro* to a shift in the microflora from Gram-positive organisms to Gram-negative anaerobes.

Gram-negative anaerobes are known to produce volatile sulphur compounds (VSCs) from blood products. Species such as *Treponema denticola*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus* and *Fusobacterium nucleatum* have been shown to produce hydrogen sulphide and methyl mercaptan from cysteine and methionine found in salivary proteins respectively (Persson *et al*, 1990). It has recently been hypothesized that the tongue dorsum may be an important area of microbial metabolism causing putrefaction, leading to halitosis (Bosy *et al*, 1994). This is due to the fact that the tongue has a large surface area supporting a high number of oral bacteria that are exposed to nutrient sources.

De Boever and Loesche (1995) investigated tongue characteristics and the production of halitosis. Correlations between VSC levels, periodontal parameters, tongue surface anatomy, the presence of trypsin-like (BANA test) activity of organisms on the tongue and bacteriological parameters were investigated. It was shown that mouth odour correlated with the amount of coating present on the tongue, was inversely related to periodontal probing depths and correlated with the presence of fissures and BANA-positive bacteria on the tongue. The presence of deep fissures may allow the development of an environment that has low levels of oxygen and is free from saliva flushing, therefore favouring a more anaerobic niche sustaining anaerobic flora.

A link between Gram-negative anaerobes and the production of hydrogen sulphide and methyl mercaptan has been reported (McNamara *et al*, 1972; Persson *et al*, 1990). The purpose of this study was to use microbiological culture to identify the principal anaerobic species present on the tongue dorsum of subjects with halitosis and of healthy controls.

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Materials and methods

Subjects

Twenty-one subjects with halitosis and 20 healthy subjects were recruited to the study. This classification was accomplished on the basis of organoleptic assessment and measurement of oral malodour using a Halimeter® (Interscan Corp., Chatsworth, CA, USA). A Halimeter® reading of at least 200 ppb or an organoleptic score of 2 or 3 was indicative of halitosis, whereas a Halimeter® reading of < 200 ppb or an organoleptic score of 0 or 1 indicates that halitosis was not present.

Tongue sampling

A sample was taken from the dorsum tongue anterior to the circumvallate papillae using a sterile brush, and then placed in 1 ml of fastidious anaerobe broth (FAB). Samples were immediately transported to the microbiology laboratory for analysis.

Microbiological culture

Samples were vortex mixed for 30 s and serial 10-fold dilutions from neat to 10^{-7} were made in freshly prepared FAB. Samples were spiral plated to fastidious anaerobe agar (FAA) plates and vancomycin anaerobic agar (VAA) plates. All plates were incubated in anaerobic conditions (80% nitrogen, 10% carbon dioxide, 10% hydrogen) in a Don Whitley MK 3 anaerobic chamber (Don Whitley Scientific Limited, Shipley, UK) at 37°C for 10 days. Following incubation, plates were examined and morphologically distinct colonies subcultured to FAA plates. Strict anaerobes were detected by their sensitivity to metronidazole, and all colonies were subjected to Gram staining. Isolated bacteria were identified to genus level on the basis of enzymatic activities and sugar fermentation characteristics (rapid ID 32A; bioMérieux, Basingstoke, UK).

Results

Tables 1 and 2 show the combined incidence of bacterial genus identified from FAA and VAA plates for each control and halitosis sample. The most prevalent species present on the tongue of both control and halitosis patients were *Veillonella*, *Prevotella* and *Fusobacterium*. Bacteria from the *Vibrio* genus and unidentifiable Gram-negative and Gram-positive anaerobes were more commonly found on the tongue of halitosis patients than healthy subjects.

Discussion

In the light of the fact that Gram-negative bacteria are thought to be the principal group of bacteria associated with oral malodour production in halitosis patients, this study only sought to identify strict anaerobes. The most commonly identified species from the tongue in both halitosis and normal subjects were *Veillonella*, *Prevotella*, *Fusobacterium*. However, Gram-negative anaerobes were identified in both control subjects and those with halitosis, and therefore cannot be solely attributed to causing halitosis. No clear correlation was evident between any specific bacterial genus and halitosis. Hartley *et al* (1996) identified *Actinomyces*, *Veillonella* and *Porphyromonas/Prevotella* as the predominant bacterial genera in most subjects with and without halitosis, results that are in good agreement with those presented in the current study.

Of significance may be the numbers of fastidious, uncultivable and novel species not detectable by this

Table 1 Bacteria isolated from control samples

HAL	*OGL*	*V*	*P*	*F*	*AS*	*Pb*	*E*	*A*	*Po*	*B*	*Pp*	*Vi*	*C*	*A–cb*	*A–r*	*A+r*	*Gv*
82	1	*												*			
86	1	*	*	*	*										*		
87	1	*	*									*		*	*		
93	1		*	*	*	*	*								*		
96	1	*	*			*			*								
98	1	*	*											*			
106	1	*		*													
109	1	*	*	*						*					*	*	
111	1	*	*	*			*			*	*						
112	1	*			*							*					
115	1	*	*	*	*	*	*					*		*		*	*
123	1	*	*	*		*	*								*		
129	1	*	*	*	*												
130	1	*	*	*													
146	1	*	*	*											*		
155	1	*			*									*			
156	1	*	*	*	*		*		*			*	*			*	
173	1	*	*	*										*	*		
189	1	*	*														
196	1	*	*	*	*	*								*	*		

HAL, Halimeter score; OGL, organoleptic score; V, *Veillonella* sp.; P, *Prevotella* sp.; F, *Fusobacterium* sp.; AS, anaerobic streptococci; Pb, *Propionibacterium* sp.; E, *Eubacterium* sp.; A, *Actinomyces* sp.; Po, *Porphyromonas* sp.; B, *Bacteroides* sp.; Pp, *Peptostreptococcus* sp.; Vi, *Vibrio* sp.; C, *Capnocytophaga* sp.; A–cb, anaerobic Gram-negative coccobacilli; A–r, anaerobic Gram-negative rod; A+r, anaerobic Gram-positive rod; Gv, Gram variable species.

Table 2 Bacteria isolated from halitosis samples

HAL	*OGL*	*V*	*P*	*F*	*AS*	*Pb*	*E*	*A*	*Po*	*B*	*Pp*	*Vi*	*C*	*A–cb*	*A–r*	*A+r*	*Gv*
130	2	*	*	*		*											
155	2	*															
173	2	*	*											*	*	*	
179	2	*	*	*	*										*	*	
180	2	*	*		*	*						*			*	*	
187	2	*	*	*	*	*	*					*		*			
193	2	*	*	*	*		*					*		*	*	*	
194	2	*	*	*	*							*		*	*		
207	2	*	*				*							*	*	*	
238	2			*	*							*				*	*
261	2	*										*		*			
285	2	*	*			*								*	*	*	
294	2	*										*		*	*		
314	2	*		*	*	*								*	*		
318	2	*	*				*					*			*	*	
327	2	*	*		*	*						*		*	*		
335	2	*	*	*		*						*					
368	2	*		*	*									*	*		
414	2	*	*	*	*									*	*		
496	2	*	*	*	*									*	*		
936	3	*	*	*	*			*		*				*		*	

HAL, Halimeter score; OGL, organoleptic score; V, *Veillonella* sp.; P, *Prevotella* sp.; F, *Fusobacterium* sp.; AS, anaerobic streptococci; Pb, *Propionibacterium* sp.; E, *Eubacterium* sp.; A, *Actinomyces* sp.; Po, *Porphyromonas* sp.; B, *Bacteroides* sp.; Pp, *Peptostreptococcus* sp.; Vi, *Vibrio* sp.; C, *Capnocytophaga* sp.; A–cb, anaerobic Gram-negative coccobacilli; A–r, anaerobic Gram-negative rod; A + r, anaerobic Gram-positive rod; Gv, Gram variable species.

study but which may be present in the samples. As both control and halitosis tongue samples have a similar incidence of known cultivable species, unidentifiable or uncultivable species may play a significant role either individually or in synergy. The role of culture-independent techniques would be essential in identifying fastidious, uncultivable and novel species. As it has been reported that approximately 50% of the oral flora is uncultivable (Socransky *et al*, 1963) and that molecular-based studies have identified a greater range of bacterial species than by culture-dependent studies alone (Hugenholtz *et al*, 1998), it seems likely that uncultivable or novel species may play a significant role in the aetiology of halitosis. Further culture-independent studies are underway in our laboratory to assess the potential importance of such species in halitosis.

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ORIGINAL ARTICLE

Clinical efficacy of a triclosan/copolymer/NaF dentifrice and a commercially available breath-freshening dentifrice on hydrogen sulfide-forming bacteria

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OBJECTIVE: The objective of this randomized, cross-over clinical study was to compare the 4-h and overnight effects of a triclosan/copolymer/fluoride (TCF-AF) dentifrice and a commercially available breath freshening (CBF) dentifrice containing fluoride for their ability to control H_2S-forming bacteria.

METHODS: Following a 1 week washout period of brushing with a regular fluoride dentifrice, subjects reported to the clinical site without performing oral hygiene, eating or drinking in preparation for baseline saliva sampling. After providing a baseline saliva sample, subjects were randomly assigned a test dentifrice and instructed to brush their teeth for 1 min, twice a day for 1 week. On the morning of the eighth day, subjects returned to the test site having refrained from oral hygiene, eating and drinking for overnight sampling. Subjects then brushed for 1 min with the assigned dentifrice, and returned for 2 and 4 h postbrushing evaluations. Following a second 1 week washout, subjects repeated the same regimen, but now using the other dentifrice. Oral microflora samples were collected by subjects rinsing with 10 ml of sterile water for 10 s. Each collected sample was serially diluted and plated in duplicate onto lead acetate agar. After incubating for 72 h, dark colonies were counted, expressed as log colony-forming units/ml, and reductions from baseline were calculated.

RESULTS: Compared with baseline, the TCF-AF dentifrice reduced H_2S-forming bacteria 0.82, 0.80 and 0.22 log units at the 2 and 4 h and overnight time points compared with 0.60, 0.43 and 0.07 log units for the CBF dentifrice. The TCFAF dentifrice was statistically significantly better ($P < 0.05$) than the CBF dentifrice at 4 h and overnight after brushing the teeth.

CONCLUSION: The results of this randomized study indicate that the TCF-AF dentifrice is effective in controlling H_2S-producing bacteria.

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Keywords: copolymer; malodor causing bacteria; triclosan; salivary microflora; Total® *Advanced Fresh*™

Introduction

The objective of this double blind clinical study was to compare the 4-h and overnight effects of a triclosan/copolymer/fluoride dentifrice with a high impact flavor (TCF-AF) and a commercially available breath freshening (CBF) dentifrice containing fluoride for their ability to control H_2S-forming bacteria.

Materials and methods

Products tested

The products tested were Colgate® Total Advanced Fresh toothpaste (Colgate-Palmolive Co., New York, NY, USA) containing 0.3% triclosan, 2% copolymer and 0.243% NaF in a silica base and Crest® plus Scope (Proctor & Gamble Co., Cincinnati, OH, USA) containing 0.243% NaF in a silica base.

Experimental design

Healthy adult volunteers, ages 21–48 years, who met entrance criteria and signed the informed consent form, were entered into the study. A randomized, double-blind clinical design with a two-treatment cross-over phase compared the effect of the two dentifrices. Prior to beginning the study, participants were required to washout the effects of previously used oral products by brushing with a regular fluoridated dentifrice for 7 days. This washout phase was followed by a treatment phase in which, after brushing with the test dentifrice for 1 week, salivary micro-flora samples were collected and plated onto media selective for H_2S-producing bacteria (Figure 1).

Clinical procedure

The subjects reported to the designated clinical study site on the morning of the baseline salivary collection without oral hygiene, eating or drinking. The subjects provided baseline saliva samples by rinsing for 10 s with 10 ml of sterile water. Subjects were then issued a

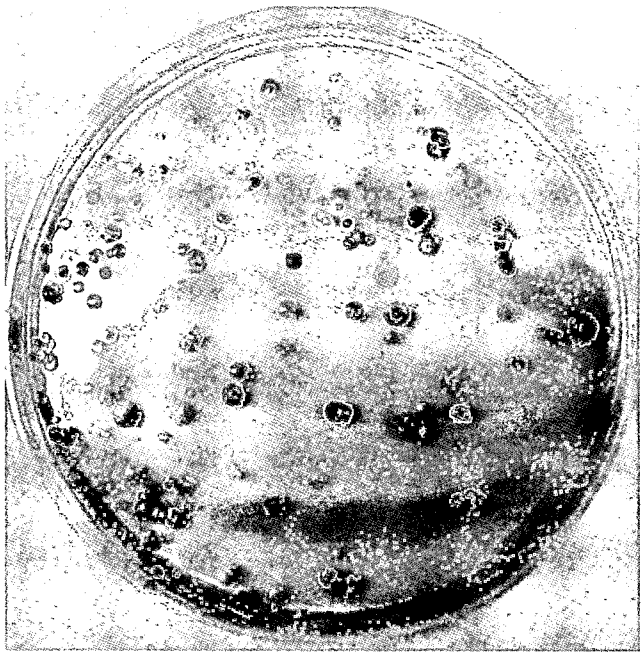

Figure 1 Agar plate with dark pigmented H_2S-producing bacterial colonies

randomly assigned treatment product and instructions for use over the next 7 days, brushing twice a day for 1 min. After the final nighttime treatment, the subjects returned to the test site in the morning without oral hygiene, eating, or drinking to provide the overnight sample. Subjects then brushed with the assigned dentifrice, and returned for 2- and 4-h post-treatment evaluations. During this 4-h period, subjects were asked to refrain from eating or drinking. After a 1-week washout period of brushing with a regular fluoridated dentifrice, the treatment regimen was repeated for the second product.

Microflora analysis

Samples collected at baseline, and the overnight, 2- and 4-h time points were serially diluted in phosphate buffered saline and plated, in duplicate, onto lead acetate agar (Anaerobe Systems, Morgan Hill, CA, USA). The plates were incubated anaerobically for a minimum of 96 h at 37°C. This method has been used to demonstrate the presence of specific bacteria in subjects with oral malodor (Pitts *et al*, 1981; El-Halabi *et al*, 1999). The dark pigmented colony forming units (cfu) were counted, converted to log units and averaged and reduction in salivary micro-flora *vs* baseline was calculated for each of the products.

Table 1 Summary of baseline bacterial counts (log cfu) for subjects who completed the study

Treatment	*n*	*Baseline counts, mean ± s.d. (log cfu)*	*Between treatment comparison*[a]
TCF-AF dentifrice	30	5.94 ± 0.45	NS
CBF dentifrice	30	5.90 ± 0.44	

[a]Significance of ANOVA comparison of baseline means.
NS = $P > 0.05$.

Statistical methods

The numbers of salivary bacteria were expressed as log cfu per plate at the baseline and postbrushing sampling time points. The mean log cfu were tabulated for each product at each time point. For each product, a paired *t*-test was performed to compare 2 h, 4 h, and overnight postuse time points to baseline. For all postbrushing time points, an analysis of covariance (ANCOVA) was performed on the scores, with the corresponding baseline as covariate and with subject and product as effects. Treatments were declared statistically significantly different if $P \leq 0.05$.

Results

Table 1 presents a summary of the baseline H_2S-producing bacteria levels. There was no statistically significant difference between the two treatments. The summary of the salivary H_2S-producing bacteria levels at the various time points measured after using TCF-AF and a commercially-available breath freshening dentifrice is presented in Table 2. Compared with baseline, the TCF-AF dentifrice significantly reduced H_2S bacteria at all measured time points; whereas the CBF dentifrice was effective at the 2 and 4-h time points. The TCF-AF dentifrice reduced H_2S-forming bacteria 0.82, 0.80 and 0.22 log units at the 2 and 4 h and overnight time points versus baseline; whereas the commercially-available breath freshening dentifrice reduced H_2S-forming bacteria 0.60, 0.43 and 0.07 log units (Table 2). Importantly, the TCF-AF dentifrice was significantly better than the commercially-available

Table 2 Summary of bacterial counts (log cfu) evaluated day 8 for subjects who completed the study

Evaluation	*Treatment*	*n*	*Bacterial count, mean ± s.d. (log cfu)*	*Reduction from baseline*[a]	*Between treatment comparison*[b]
2 h	TCF-AF dentifrice	30	5.12 ± 0.44	0.82	$P > 0.05$
	CBF dentifrice	30	5.30 ± 0.39	0.60	
4 h	TCF-AF dentifrice	30	5.14 ± 0.41	0.80	$P < 0.05$
	CBF dentifrice	30	5.47 ± 0.44	0.43	
Overnight	TCF-AF dentifrice	30	5.72 ± 0.43	0.22	$P < 0.05$
	CBF dentifrice	30	5.84 ± 0.42	0.07	

[a]Within treatment reduction between baseline and post-treatment counts.
[b]Significance of ANOVA comparison of baseline adjusted mean bacterial scores.

fresh breath product after 4 h and overnight (Table 2).

Discussion

A new triclosan/copolymer/fluoride toothpaste variant with a high impact breath freshening flavor was effective in reducing H_2S-producing bacteria 2 and 4 h and overnight. The formula was more effective than a commercially-available breath freshening dentifrice at 4 h and overnight after brushing the teeth. The results of this randomized study indicate that the TCF-AF dentifrice is effective in controlling H_2S-producing bacteria.

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ORIGINAL ARTICLE

Quantitative detection of volatile sulfur compound-producing microorganisms in oral specimens using real-time PCR

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OBJECTIVE: It is well-known that some periodontopathic bacteria, especially *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Tannerella forsythia* (formerly *Bacteroides forsythus* or *Tan. forsythensis*), and *Treponema denticola*, actively produce volatile sulfur compounds (VSCs), such as H_2S and CH_3SH. We previously reported a qualitative relationship between periodontopathic bacteria and VSCs; however, a quantitative analysis of periodontopathic bacteria in oral specimens is required for further characterization of the relationship between oral bacteria and VSCs. In this study, we report a real-time polymerase chain reaction (PCR) method for the quantitative analysis of VSC-producing bacteria in oral specimens.

SUBJECTS AND METHODS: Specimens were collected from 22 patients who visited the Preventive Dentistry and Breath Odor Clinic of Kyushu Dental College. A real-time PCR assay using the TaqMan system, based on the 5′–3′ exonuclease activity of *Taq* polymerase, was employed for the quantitative analysis of periodontopathic bacteria that produce VSCs.

RESULTS: Using real-time PCR, we performed a quantitative analysis of *P. gingivalis*, *F. nucleatum*, *Tan. forsythia*, and *T. denticola* in the saliva, on the tongue coat, and in the subgingival plaque of patients with oral malodor.

CONCLUSION: Real-time PCR using the TaqMan system can be used for the quantitative analysis of VSC-producing oral bacteria.

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Keywords: halitosis; real-time polymerase chain reaction; quantitative detection; periodontopathic bacteria; volatile sulfur compounds

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Introduction

It is well-known that specific gram-negative bacteria, which colonize the subgingival area, are the major oral bacteria that produce volatile sulfur compounds (VSCs) (Rosenberg, 1997). *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Tannerella forsythia* (formerly *Bacteroides forsythus* or *Tan. forsythensis*), which are gram-negative anaerobic rods, actively produce VSCs such as H_2S and CH_3SH. *Treponema denticola*, which is a helical microorganism, also produces VSCs (Persson *et al*, 1990). In addition to these periodontopathic bacteria, both *Peptostreptococcus* sp. and *Streptococcus salivarius* produce H_2S (Rosenberg, 1997). Several methods have been developed to identify these microorganisms, many of which are polymerase chain reaction (PCR)-based bacterial detection systems. Most of the reported PCR-based diagnostic systems are qualitative analysis methods (Awano *et al*, 2002) and are therefore unsuitable for the accurate evaluation of bacteria causing oral malodor. Quantitative analysis is essential for monitoring the cell number and ratio of bacteria in oral specimens from the saliva, tongue coat, and subgingival plaque (Yoshida *et al*, 2003; Kuboniwa *et al*, 2004; Suzuki *et al*, 2004).

A real-time PCR assay using the TaqMan system, which is based on the 5′–3′ exonuclease activity of *Taq* polymerase, has been developed for the quantitative detection of DNA copy number. Briefly, an oligonucleotide probe with a reporter fluorescent dye attached to its 5′-end and a quencher dye attached to its 3′-end is designed to hybridize to the target gene. During PCR amplification, the quencher dye of the probe is cleaved by the 5′-nuclease activity of *Taq* polymerase, resulting in the accumulation of reporter fluorescence. The release of the fluorescent dye during amplification allows for the rapid detection and quantification of DNA.

This study describes a real-time PCR method using TaqMan for the relative quantification of oral malodor-associated microorganisms, including *P. gingivalis*, *F. nucleatum*, *Tan. forsythia*, and *Tre. denticola*, from oral specimens such as saliva, tongue coat, and subgingival plaque.

Materials and methods

Subjects

Twenty-two subjects [nine males and 13 females; age, 44.9 ± 15.0 years (mean ± s.d.); age range: 23–71] who visited the Preventive Dentistry and Breath Odor Clinic of Kyushu Dental College were evaluated.

Preparation of saliva, tongue coat, and subgingival plaque samples

Human saliva, tongue coat, and subgingival plaque samples were prepared as follows. Equal volumes (500 μl) of saliva and phosphate-buffered saline (PBS; 0.12 M NaCl, 0.01 M Na_2HPO_4, 5 mM KH_2PO_4, pH 7.5) were mixed and centrifuged at 12 000 *g* for 10 min. The precipitate was mixed with 500 μl of cell lysis buffer [1.0% Triton X-100, 20 mM Tris-HCl, 2 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0] using a vortexer, and the chromosomal DNA from the bacteria was extracted by boiling at 100°C for 10 min. The saliva was removed from the tongue coat using a pressurized air jet stream and a sample was taken from the dorso-posterior surface of the tongue with a sterile cotton ball. About 1 ml (wet weight) of tongue coat material was washed twice with PBS. The precipitate was suspended in 100 μl of cell lysis buffer, the lysate was boiled at 100°C for 10 min, and the chromosomal DNA was extracted. Subgingival plaque samples were obtained by inserting a sterile endodontic paperpoint into the subgingival site for 10 s. The paperpoint was transferred into 200 μl PBS and centrifuged at 12 000 *g* for 5 min. The cells were resuspended in 100 μl of cell lysis buffer, the lysate was boiled at 100°C for 5 min, and the supernatant was used as PCR template.

Halitosis assessment using gas chromatography

The H_2S and CH_3SH concentrations in mouth air were determined using a gas chromatograph (G2800, Yanaco, Kyoto, Japan) equipped with a flame photometric detector and a 3.4 mm × 3-m glass column packed with 25% 1,2,3-Tris (2-cyanoethoxy) propane in chromosorb W (AW-DMCS, 60/80 mesh). The column conditions were as follows: column temperature, 60°C; injection port temperature, 120°C; flame photometric detector temperature, 120°C; nitrogen gas flow pressure, 1.2 kg cm^{-2}; hydrogen gas flow pressure, 1.0 kg cm^{-2}; and air flow pressure, 1.0 kg cm^{-2}.

Real-time PCR detection

Initially, conventional PCR was used to detect the presence of *P. gingivalis*, *F. nucleatum*, *Tan. forsythia*, and *T. denticola* in the saliva, on the tongue coat, and in the subgingival plaque of each subject. Then the specimens from these patients were analyzed using TaqMan and real-time PCR. For each real-time PCR, 20 μl of a mixture containing 5 μl of lysed cells, 10× LightCycler™ FastStart DNA Master Hybridization Probes (Roche Diagnostics, Mannheim, Germany), sense and antisense primers at a concentration of 500 nM each, and 200 nM TaqMan probe was placed in a capillary tube. The species-specific primers and TaqMan probes for real-time PCR are shown in Table 1. Amplification and detection were performed with the LightCycler Sequence Detection System (Roche Diagnostics). For the relative quantification, the copy numbers of periodontopathic bacterial genes were normalized to the copy number of the 16S rRNA gene using the simplified comparative threshold cycle (ΔCt) method (Yoshida *et al*, 2003). Briefly, the fold-difference (N) in the copy number of a periodontopathic bacteria-specific gene relative to the copy number of the 16S rRNA gene was determined as:

$$N = 2^{\Delta Ct} = 2^{(Ct\text{periodontopathicbacteria} - Ct16\text{SrRNA})}$$

The ΔCt values for the sample and for the 16S rRNA were determined by subtracting the average Ct value for the target gene from the average Ct value for the 16S rRNA gene.

Table 1 Oligonucleotide primers and probes used for real-time PCR

Designation	*Sequence*	*Amplicon size (bp)*	*Target gene*
Primers			
Pg1198-F	5′-TACCCATCGTCGCCTTGGT-3′	126	16s rRNA
Pg1323-R	5′-CGGACTAAAACCGCATACACTTG-3′		
Fn619-F	5′-CGCAGAAGGTGAAAGTCCTGTAT-3′	99	16S rRNA
Fn719-R	5′-TGGTCCTCACTGATTCACACAGA-3′		
Bf18-F	5′-ATCCTGGCTCAGGATGAACG-3′	225	16S rRNA
Bf243-R	5′-TACGCATACCCATCCGCAA-3′		
Td1394-F	5′-AGAGCAAGCTCTCCCTTACCGT-3′	105	16S rRNA
Td1498-R	5′-TAAGGGCGGCTTGAAATAATGA-3′		
Uni152-F	5′-CGCTAGTAATCGTGGATCAGAATG-3′	69	16S rRNA
Uni220-R	5′-TGTGACGGGCGGTGTGTA-3′		
Fluorescent probes			
Pg1238T	5′-FAM-TCGAGTATTCCTCAAGCATTCTCGCACG-TAMRA-3′		16S rRNA
Fn663T	5′-FAM-ACTTTGCTCCCAAGTAACATGGAACACGAG-TAMRA-3′		16S rRNA
Bf124T	5′-FAM-ATGTAACCTGCCCGCAACAGAGGGATAAC-TAMRA-3′		16S rRNA
Td1444T	5′-FAM-CAGCGTTCGTTCTGAGCCAGGATCA-TAMRA-3′		16S rRNA
Uni177T	5′-FAM-CACGGTGAATACGTTCCCGGGC-TAMRA-3′		16S rRNA

Pg, *Porphyromonas gingivalis*; Fn, *Fusobacterium bacterium*; Bf, *Tannerella forsythia*; Td, *Treponema denticola*; Uni, universal primer; PCR, polymerase chain reaction; rRNA, ribosomal ribonucleic acid.

Results and discussion

The detection and percentages of periodontopathic bacteria in the oral specimens of all subjects are shown in Tables 2–4. The percentages of *P. gingivalis*, *F. nucleatum*, and *T. denticola* in each saliva sample varied by a few orders of magnitude (Table 2). *Tannerella forsythia* was not detected in saliva samples by real-time PCR. Similar results were obtained with the tongue coat samples, in which the proportions of bacteria ranged from 0 to 3.65×10^{-2} for *P. gingivalis*, 0 to 3.37 for *F. nucleatum*, and 0 to 1.21×10^{-3} for *T. denticola*.

Table 2 Detection and percentages of periodontopathic bacteria in the saliva

Sample and patient number	*Conventional PCR results*				*Real-time PCR results (%)*			
	P. gingivalis	*F. nucleatum*	*T. forsythia*	*T. denticola*	*P. gingivalis*	*F. nucleatum*	*T. forsythia*	*T. denticola*
1	−	+	−	−	−	5.02×10^{-2}	−	−
2	+	−	−	+	9.67×10^{-5}	−	−	2.59×10^{-4}
3	−	+	−	+	−	1.30	−	3.80×10^{-2}
4	−	+	−	+	−	2.50×10^{-1}	−	6.01×10^{-2}
5	−	+	−	−	−	1.55×10^{-2}	−	−
6	−	−	−	−	−	−	−	−
7	+	+	−	−	1.30	3.76×10^{-4}	−	−
8	−	−	−	−	−	−	−	−
9	−	−	−	−	−	−	−	−
10	−	−	−	−	−	−	−	−
11	−	+	−	−	−	7.49×10^{-1}	−	−
12	−	−	−	−	−	−	−	−
13	+	+	−	−	1.59×10^{-1}	1.93×10^{-1}	−	−
14	−	−	−	−	−	−	−	−
15	−	−	−	−	−	−	−	−
16	−	−	−	−	−	−	−	−
17	−	−	−	−	−	−	−	−
18	−	+	+	−	−	−	−	−
19	−	−	−	−	−	−	−	−
20	−	−	−	−	−	−	−	−
21	−	−	−	−	−	−	−	−
22	+	−	−	−	5.13×10^{-2}	−	−	−

Porphyromonas gingivalis; *Fusobacterium nucleatum*; *Tannerella forsythia*; *Treponema denticola*; PCR, polymerase chain reaction.

Table 3 Detection and percentages of periodontopathic bacteria on the tongue coat

Sample and patient number	*Conventional PCR results*				*Real-time PCR results (%)*			
	P. gingivalis	*F. nucleatum*	*T. forsythia*	*T. denticola*	*P. gingivalis*	*F. nucleatum*	*T. forsythia*	*T. denticola*
1	−	+	−	−	−	1.81×10^{-2}	−	−
2	+	+	−	+	1.10×10^{-4}	2.02×10^{-2}	−	1.21×10^{-3}
3	−	+	−	−	−	9.74×10^{-5}	−	−
4	−	+	−	+	−	3.37	−	4.47×10^{-4}
5	−	+	+	−	−	1.37	−	−
6	+	+	−	−	−	1.26×10^{-2}	−	−
7	+	−	−	−	3.65×10^{-2}	−	−	−
8	−	−	−	−	−	−	−	−
9	−	−	−	−	−	−	−	−
10	+	+	−	−	5.65×10^{-7}	3.12×10^{-4}	−	−
11	−	+	−	−	−	3.99×10^{-1}	−	−
12	−	+	−	−	−	1.69×10^{-2}	−	−
13	+	−	−	−	−	−	−	−
14	−	+	−	−	−	2.72	−	−
15	−	−	−	−	−	−	−	−
16	−	−	−	−	−	−	−	−
17	−	+	−	−	−	3.85×10^{-1}	−	−
18	−	+	−	−	−	1.16×10^{-8}	−	−
19	−	+	−	−	−	1.61×10^{-3}	−	−
20	−	+	−	−	−	3.69×10^{-5}	−	−
21	+	+	−	−	7.95×10^{-4}	2.72×10^{-2}	−	−
22	+	+	−	−	1.14×10^{-5}	5.61×10^{-3}	−	−

Porphyromonas gingivalis; *Fusobacterium nucleatum*; *Tannerella forsythia*; *Treponema denticola*; PCR, polymerase chain reaction.

Table 4 Detection and percentages of periodontopathic bacteria in the subgingival plaque

Sample and patient number	*Conventional PCR results*				*Real-time PCR results (%)*			
	P. gingivalis	*F. nucleatum*	*T. forsythia*	*T. denticola*	*P. gingivalis*	*F. nucleatum*	*T. forsythia*	*T. denticola*
1	+	+	−	+	8.36×10^{-5}	6.18×10^{-2}	−	2.97×10^{-3}
2	+	+	−	+	1.72×10^{-2}	3.54×10^{1}	−	3.26
3	−	+	+	+	−	−	1.41	1.67×10^{-1}
4	−	+	+	+	−	1.57	4.52×10^{-2}	1.12×10^{1}
5	+	+	−	−	−	3.67×10^{-1}	−	−
6	+	+	−	−	5.34×10^{-1}	3.38×10^{-1}	−	−
7	+	+	−	+	1.22×10^{-6}	9.58×10^{-5}	−	2.74×10^{-3}
8	+	+	−	−	−	5.01×10^{-1}	−	−
9	+	+	−	−	−	8.14×10^{-1}	−	−
10	+	+	−	−	−	7.01×10^{-5}	−	−
11	+	+	−	−	5.30×10^{-1}	8.55×10^{-1}	−	−
12	+	+	−	+	2.44×10^{-2}	−	−	2.28×10^{1}
13	+	+	−	+	−	−	−	1.06×10^{1}
14	−	−	−	−	−	−	−	−
15	+	+	−	+	1.92×10^{-5}	2.35×10^{-4}	−	3.15×10^{-5}
16	+	+	−	−	6.80×10^{-1}	3.61	−	−
17	−	+	+	−	−	1.34×10^{-3}	−	−
18	−	+	+	−	−	1.29×10^{-5}	3.47×10^{-6}	−
19	+	+	−	+	9.09×10^{-5}	1.80×10^{1}	−	1.48
20	+	+	−	+	2.31×10^{-6}	1.29×10^{-5}	−	5.53×10^{-2}
21	+	+	+	+	3.79×10^{1}	−	−	1.94
22	+	+	−	+	−	7.16×10^{-3}	−	−

Porphyromonas gingivalis; *Fusobacterium nucleatum*; *Tannerella forsythia*; *Treponema denticola*; PCR, polymerase chain reaction.

Table 5 Representative profiles of patients with halitosis

Patient (number)	*Gender*	*Age*	*Levels of VSCs (ng ml^{-1})*		*Sample*	*Bacterial occurrence (%)*			
			H_2S	*CH_3SH*		*P. gingivalis*	*F. nucleatum*	*T. forsythia*	*T. denticola*
12	Female	67	1.342	0.744	Saliva	−	−	−	−
					Tongue coat	−	1.69×10^{-2}	−	−
					Subgingival plaque	2.44×10^{-2}	−	−	2.28×10^{1}
13	Female	32	0.347	0.050	Saliva	1.59×10^{-1}	1.93×10^{-1}	−	−
					Tongue coat	−	−	−	−
					Subgingival plaque	−	−	−	1.06×10^{1}
18	Male	24	0.323	0.076	Saliva	−	−	−	−
					Tongue coat	−	1.16×10^{-8}	−	−
					Subgingival plaque	−	1.29×10^{-5}	3.47×10^{-6}	−

Porphyromonas gingivalis; *Fusobacterium nucleatum*; *Tannerella forsythia*; *Treponema denticola*; VSC, volatile sulfur compounds.

Tannerella forsythia was not detected in tongue coat samples in this study (Table 3). The percentages of *P. gingivalis*, *F. nucleatum*, *Tan. forsythia*, and *T. denticola* in each subgingival plaque sample varied by several orders of magnitude (Table 4). We detected *F. nucleatum* in 21 of 22 (95%) periodontal sites. Our result is similar to that of a previous study (Suzuki *et al*, 2004). In our study, the detection frequencies of *F. nucleatum* in saliva and the tongue coat were lower than in subgingival plaque. The proportion of bacteria ranged from 0 to 3.79×10^{1} for *P. gingivalis*, 0 to 3.54×10^{1} for *F. nucleatum*, 0 to 1.41×10^{-2} for *Tan. forsythia*, and 0 to 2.28×10^{-2} for *T. denticola*. It is necessary in future work to focus on the production of H_2S by *Peptostreptococcus* and *S. salivarius*. A quantitative analysis of *Peptostreptococcus* and *S. salivarius* will be meaningful for analyzing the relationship between VSC and the presence of these organisms in the context of the etiology of oral malodor. Representative VSC patterns and percentages of VSC-producing bacteria are shown in Figure 1 and Table 5.

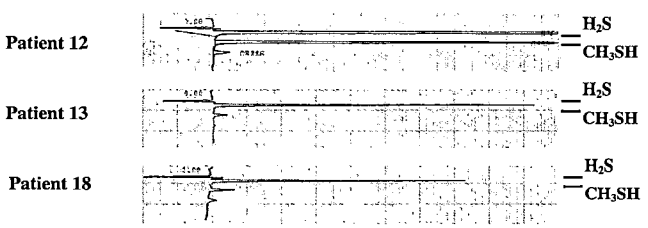

Figure 1 Gas chromatographs of mouth air samples from patients with oral malodor. Patient numbers correspond to the patient numbers shown in tables

In conclusion, using real-time PCR, we performed a quantitative analysis of *P. gingivalis*, *F. nucleatum*, *Tan. forsythia*, and *T. denticola* in the saliva, the tongue coat, and the subgingival plaque of patients at a Breath Odor Clinic. We will continue to study whether this assay system is useful for analyzing the relationship between oral bacteria and halitosis.

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ORIGINAL ARTICLE

Association between oral malodour and periodontal disease-related parameters in a population of 71 Israelis

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The aim of the present study was to determine whether oral malodour and periodontal disease parameters are associated with one another in 71 Israeli subjects (mean age 36.2 ± 18.4; ages ranging from 15 to 65). Parameters measured included whole mouth odour judge scoring, Halimeter, OK to Kiss test, gingival index, plaque index and probing depth. Odour judge scores were significantly associated with Halimeter® ($r = 0.55$; $P < 0.001$), as well as the OK to Kiss test ($r = 0.52$; $P < 0.001$). However, neither gingival index, plaque index nor probing depth was significantly associated with odour judge scores or Halimeter® scores. Logistic regression analysis showed that both Halimeter® and OK to Kiss scores factored significantly ($P = 0.005$ and 0.018, respectively, odds ratios 14.9 and 2.7, respectively) in predicting the severity of oral malodour. Results suggest that in the population studied, periodontal health and oral malodour are not associated with one another.
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Keywords: oral malodor; periodontal disease; organoleptic tests; halimeter; dental tests;

Introduction

Bad breath is a common human problem. It originates, in the large majority of cases, within the mouth itself (Tonzetich, 1978; Rosenberg, 2002). For over 60 years, many researchers have, based on various experiments, deduced that periodontal diseases cause bad breath (Sulser *et al*, 1939). These studies were recently extensively reviewed (Morita and Wang, 2001). Nevertheless, in an extensive study involving over 120 Canadian subjects, periodontal disease parameters and oral malodour measurements were not significantly associated with one another (Bosy *et al*, 1994). The purpose of the present investigation was to test for a relationship between oral malodour and periodontal parameters in a group of 71 Israeli adults. The data obtained support the results of Bosy *et al* (1994), i.e. that oral malodour and periodontal disease parameters tested are not directly related.

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Materials and methods

Study sample and data gathering procedures
Study population
A convenience sample of 71 consenting Israeli subjects, ages ranging from 15 to 65 (mean age 36.2 ± 18.4) who were either undergoing a standard medical ($N = 14$) checkup, 12 patients who were about to commence orthodontic treatment at Tel Aviv University, and 55 subjects who were siblings, or parents of orthodontic patients at Tel Aviv University. Breath odour and dental tests were performed between 9.00 AM and 1.00 PM, following a 2 h fast. Volunteers filled out an informed consent form and questionnaire. Exclusion criteria included people taking antibiotics, subjects with complete or removable partial dentures or with extensive fixed partial dentures or those with impacted third molars and subjects under active treatment of periodontal disease during the previous 2 months. The study was approved by the ethics review board of Tel Aviv University.

Testing of oral malodor
Organoleptic
Organoleptic testing was carried out by a trained and calibrated odour judge (ES). The subjects were asked to exhale briefly through their mouth, at a distance 10 cm away from the nose of the organoleptic judge. Malodor was graded on a scale of 0–5 as follows (Rosenberg *et al*, 1991a,b), allowing for scoring between the integers: 0 = no appreciable malodour, 1 = barely noticeable malodour, 2 = slight but clearly noticeable malodour, 3 = moderate malodour, 4 = strong malodour, 5 = extremely strong malodour.

Halimeter® (volatile sulphide monitor)
Volatile sulphides were tested using a Halimeter® (Interscan, Chatsworth, CA, USA) as previously reported (Rosenberg *et al*, 1991a,b). A plastic straw connected

to the monitor inlet via tubing was inserted approximately 4 cm into the partially open mouth of the subject while the subject inhaled and exhaled through the nose. Subjects were asked to refrain from speaking for at least 1 min prior to the test. Measurements were repeated three times and the peak p.p.b. value recorded for each trial. The logarithm (base 10) of the average of the two closest values was employed in the analysis.

OK to Kiss

This is a colour test based on the level of β-galactosidase in human saliva (Sterer *et al*, 2002; Sterer and Rosenberg, 2002) The test utilizes a small disposable plastic spittoon, with a central disc coated with chromogenic substrate. Each subjects wetted the disc with a sample of their saliva. Following 5 min incubation at room temperature, classification was recorded based on the following scale: 0 = no blue specks or coloration, 1 = extremely faint blue reaction or scattered specks of blue, 2 = stronger blue reaction (many specks or general blue coloration).

Dental tests

Periodontal probing, gingival plaque index and plaque index were assessed using Ramfjord index teeth as reported by Kozlovsky *et al* (1994). In each instance, the average score for each tooth was calculated and then summed and divided by the number of teeth examined ($N = 6$).

Results

Pearson correlations comparing oral malodour and dental parameters are summarized in Table 1. Odour judge scores were significantly associated with Halimeter® ($r = 0.55$; $P < 0.001$), as well as the OK to Kiss test ($r = 0.52$; $P < 0.001$). Logistic regression analysis showed that both Halimeter® and OK to Kiss scores factored significantly ($P = 0.005$ and 0.018, respectively, odds ratios 14.9 and 2.7, respectively) in predicting the severity of odour judge scores. These results support recent results suggesting that the OK to Kiss is a simple and valid test associated with oral malodour (Sterer *et al*, 2002; Sterer and Rosenberg, 2002). Both the volatile sulphur compounds (VSC) and enzymatic tests appear to measure, at least to some extent, different aspects of oral malodour, as both parameters factored significantly in logistic regression analysis predicting oral malodour levels.

In contrast to the significant correlations described above comparing oral malodour parameters, neither gingival index, plaque index, nor probing depth was significantly associated with odour judge scores or Halimeter® scores ($P > 0.05$). Whereas gingival index and plaque index scores were significantly associated with one another ($P = 0.001$), neither index was associated with probing depth ($P > 0.05$).

Discussion

To what extent is bad breath due to periodontal disease? One might cite the following in support for a relationship: (i) subgingival pockets are putrid; (ii) sulphur-containing and other volatiles (e.g. cadaverine) that may be elaborated by subgingival microbiota are toxic to gingival tissues; (iii) anaerobic Gram-negative micro-organisms produce more malodour *in vitro* than Gram-positive micro-organisms (Sulser *et al*, 1939; Kleinberg and Codipilly, 1997; Morita and Wang, 2001). However, one must consider alternative arguments: (i) mass transfer of gases from periodontal pockets may often be very low; (ii) the pockets are poorly accessible to antibacterial agents in mouthwashes, yet effective mouthwashes can reduce bad breath dramatically without concomitant reductions in gingival parameters (Rosenberg *et al*, 1991a); (iii) tongue cleaning alone brings about 70% in reduction in volatile sulphur levels, without direct involvement of the gingiva (Tonzetich, 1978).

Clinical studies comparing oral malodour and periodontal disease have produced different results. For example, Kozlovsky *et al* (1994) demonstrated highly significant ($P < 0.001$) associations between malodour scores and periodontal disease-related parameters in a group of approximately 50 Israeli adults. However, a similar study carried out by Bosy *et al* (1994) on 127 Canadian subjects, arrived at the opposite conclusion. Results, analysed using both dichotomous and regression analysis, indicated that oral malodor and periodontal parameters were not significantly associated with one another. On the contrary, mean floss odour was significantly associated not only with whole mouth malodor ($P < 0.01$), but also with gingival index ($P < 0.01$). Floss odour also factored as an independent parameter accounting for volatile sulphide levels, following the stronger associations of whole mouth malodor and tongue odour (backward stepwise regression analysis). These data suggest the possibility that interdental odour may be an independent factor linking both periodontal disease and bad breath. If this is the case, then the apparent associations often observed between these two factors in clinical studies are actually

Table 1 Pearson correlations comparing odour and dental parameters [significant results ($P < 0.05$) are indicated in bold]

	Whole mouth odour	*OK to Kiss*	*GI*	*PI*
OK to Kiss	**0.518 (0.001)**	–	–	–
Halimeter®	**0.585 (<0.001)**	**0.383 (0.001)**	−0.097 (0.416)	−0.071 (0.555)
GI	0.185 (0.123)	0.086 (0.476)	–	–
PI	0.111 (0.362)	0.088 (0.468)	**0.379 (0.001)**	–
Probing depth	−0.054 (0.654)	−0.027 (0.822)	0.166 (0.163)	−0.003 (0.982)

Values are given as *R* (*P*-value).
GI, gingival index; PI, plaque index

indirect. The results presented in this paper support the premise that oral malodour and periodontal disease levels are not directly related.

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ORIGINAL ARTICLE

Creation of oral care flavours to deliver breath-freshening benefits

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OBJECTIVE: Oral care products deliver breath freshening primarily via mechano-chemical cleaning or by antimicrobial active systems. Dental flavours provide taste benefits, and freshen breath mainly by sensorial masking. We aimed to determine whether flavours could deliver breath freshening in products by inhibiting bacterial volatile sulphide compound (VSC) production.

SUBJECTS AND METHODS: Flavour materials were screened for inhibition of hydrogen sulphide formation by *Klebsiella pneumoniae in vitro*, grouped by efficacy, and data provided to flavourists. Flavours were formulated to maximize the content of VSC-effective ingredients and re-screened to confirm performance. Extensive, iterative testing of flavours identified reliable creative rules to deliver efficient inhibition of H_2S generation. Breath-freshening flavours in whole products were then tested in-house in a 'breath freshness panel'.

MAIN OUTCOME MEASURES: Malodour of panellists (not preselected for malodour score) was scored before and after product use, on the 'Rosenberg' 0–5 scale, together with residual flavour score, by extensively trained judges. Products were tested in double-blind, crossover studies, and results analysed using ANOVA.

RESULTS AND CONCLUSIONS: Products flavoured using these rules delivered significantly greater breath freshening at 2 h than control products, and equivalent benefits to products containing 0.1% (w/w) triclosan or 0.2% (w/w) zinc sulphate.

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Keywords: halitosis; flavouring agents; dentifrice; sulphides; breath freshening

Introduction

Oral malodour (or halitosis) is a non-life-threatening but potentially psycho-socially distressing condition that is relatively common throughout the adult population (Scully *et al*, 1994). For many individuals it can be persistent and often does not result from poor oral hygiene. Estimates of prevalence vary widely, from 24% of Japanese individuals who complain of bad breath (Miyazaki *et al*, 1995) to more than 50% of the North American population (Tessier and Kulkarni, 1991; Bosy, 1997). Furthermore, the prevalence of either overt or covert 'halitophobia' increases this proportion. The potential for therapies is thus colossal, and halitosis remedies form a multimillion-dollar industry.

The great majority of halitosis results from bacterial degradation of endogenous or dietary organic compounds, to yield a melange of malodorants. The major contributors to oral malodour are sulphides (including hydrogen sulphide and methyl sulphides), a variety of nitrogenous compounds (amines, diamines, indole, etc.) and volatile fatty acids (butyrate, etc.). It has been estimated that sulphides account for approximately 60% of organoleptically measured oral malodour (Sterer *et al*, 2002).

Control of oral malodour conventionally relies on mechanical or chemical cleaning of the oral cavity. Mechanical cleaning is usually achieved by the use of dentifrices with toothbrushes, or by flossing or rinsing. A number of chemicals are incorporated into oral care products to facilitate this cleaning, including detergents such as sodium lauryl sulphate (SLS), and abrasives such as silica. These approaches are often augmented by the use of antimicrobial activities in products, such as triclosan, chlorhexidine and metal salts. In addition to these overt antimicrobial actives, various other materials incorporated into products can also deliver antimicrobial effects – for example, alcohol in mouthwashes, and SLS in toothpastes. Dental flavours are incorporated into oral care products for taste/hedonic reasons, and can deliver significant breath-freshening benefits to the consumer, principally via masking of malodours. However, flavour materials include a wide range of compounds, several of which are known to have significant, although relatively subtle, effects on microbial metabolism.

The aim of this investigation was to determine whether the modest effects of individual flavour ingredients on oral microbial metabolism could be consolidated to produce a range of dental flavours that would significantly inhibit the production of volatile sulphur

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compounds (VSC) by bacteria *in vitro*. More importantly, we aimed to determine whether the effects of these flavours *in vitro* could be translated into organoleptically perceivable breath-freshening benefits in oral care products using an in-house breath-freshening panel. Finally, we aimed to establish whether these flavours could allow the removal of antimicrobial agents from oral care products without loss of breath-freshening efficacy.

Materials and methods

Selection and in vitro *screening of flavour materials*
Flavour ingredients from the Quest flavour palette were screened in the Quest *in vitro* VSC reduction model. Ingredients were all tested at a final concentration of 500 p.p.m. (0.05% w/w). A 250 ml overnight broth culture of *Klebsiella pneumoniae* ATCC 10031 (ATCC, Rockville, MD, USA) was harvested by centrifugation at 3500 *g* for 10 min. These cells were then washed three times using sterile 0.3% Trytone Soya Broth (TSB) and resuspended in 10 ml of 0.3% TSB. The optical density of the cellular biomass was then adjusted using 0.3% TSB to give an optical density equivalent to 39 at a wavelength of 610 nm. Sterile cysteine was then added to the cellular suspension to give a concentration of 0.1% (w/v) cysteine in the reaction vessel. The mixture was then incubated at 37°C for 1 h to induce cysteine metabolism in the micro-organisms. After this time the cells were washed twice using 0.3% TSB solution to remove excess cysteine and resuspended in the same volume of 0.3% TSB solution.

The flavour or ingredient under investigation was prepared in sterile TSB solutions at concentrations of 32 600 p.p.m. (3.26% w/w). A 0.2 ml aliquot of this stock solution was mixed with 11.8 ml of sterile 0.3% (w/v) TSB solution in a sterile glass headspace bottle (volume 20 ml). To this mixture was then added 0.005 g of cysteine. This was then thoroughly mixed and incubated at 37°C for 1 h to allow the mixture to equilibrate. After this time 1 ml of the previously prepared bacterial suspension was added to the vial to give an optical density (OD) of 3 units at 610 nm, and a final concentration of flavour material of 500 p.p.m. (0.05% w/w). The solution was then capped to produce an airtight seal and incubated for 60 min at 37°C. After this time 500 μl of the headspace gas was removed and the quantity of H_2S present in the sample estimated using gas chromatographic (GC) analysis. A VSC-inhibition percentage was calculated for each flavour or flavour ingredient by comparison with an untreated control. Further to this comparison, a VSC-reduction index (VSCRI) was calculated, by dividing the ingredient or flavour percentage reduction by that achieved by standard chemical inhibitors.

Testing of breath-freshening flavours in dentifrices in the quest breath freshness panel
Breath-freshening flavour compositions were incorporated into standard toothpaste base and tested for malodour counteraction in the Quest Breath Freshness Panel. The aim of the panel was to allow objective testing of the effectiveness of toothpaste, mouthwash, chewing gum or other oral health care formulations on human-assessed (organoleptic) breath odour. The effects of products containing proposed breath-freshening flavours were compared against appropriate placebos (containing basal components, but without breath-freshening flavours).

Judge training
The judges on the Quest Breath Freshness Panel underwent an extensive training programme. This included a prescreening to include only judges scoring highly in standard Quest sniffing panel tests, including recognition, differentiation, ranking and descriptive language. Judges who passed this screening then assessed odours of representative chemicals that contribute to oral malodour including sulphides, amines and organic acids. These sniffing sessions included both descriptive and quantitative assessment of malodorants. Finally, the judges undertook organoleptic assessment of human breath odour, on the 'Rosenberg' 0–5 scale (Rosenberg *et al*, 1991a,b) (0 = no odour, 5 = severe malodour), initially on colleagues, so that informal discussion of perceived odours (both descriptive and intensity) could take place. These discussions provided feedback that allowed standardization between judges. In the last element of breath assessment, judges scored subjects 'blind' to other judges' scores, and these results were statistically evaluated to ensure that judges' scores were comparable.

Study design
The study design is summarized in Table 1. All studies were double-blind (i.e. neither judges, nor panellists knew which breath freshening or control product they were using), balanced carry-over, experimental designs, with repeated measures. A washout toothpaste was used throughout each trial and 24 subjects were used for each panel, with three judges. Panellists were required to eschew spicy foods (e.g. garlic, onions) the night before each trial day. They were also required to avoid all oral hygiene measures on the morning of the trial, and not to consume coffee or to use perfumes or cosmetics. The trials were carried out from 9 AM until 1 PM. A standard quantity (approximately 1.5 g) of a toothpaste formulation containing one of the breath-freshening flavour compositions or a standard flavour control was applied to a toothbrush, and the panellist used the product to brush

Table 1 Quest breath freshness panel – key protocol features

'Washout' toothpaste used throughout trial
No spicy food night before trial
No oral hygiene on morning of trial
No coffee, no perfumes, no cosmetics on day of trial
Trials carried out approximately from 9 AM until 1 PM
Two or three trained breath odour judges per trial
Single product use – supervised, standardized product application
Measurement of breath following 2 min with mouth closed
Breath malodour intensity measured on a 0–5 organoleptic scale
Residual flavour 'pleasantness' intensity measured, also on 0–5 organoleptic scale

the teeth in a normal fashion for 90 s. The panel members were allocated test or control products on subsequent weeks in accordance with a balanced carry-over design. Breath scores were assessed organoleptically on the 'Rosenberg' 0–5 scale (Rosenberg *et al*, 1991a,b), modified to allow the use of 1/2-point measurements, at time zero (prebrushing), and at 30, 60, 90 and 120 min after brushing. Breath assessment was carried out by asking the panellist to close their mouth for a timed, 2-min period. At the end of this period, the panellist tilted their head back and opened their mouth, but without breathing. The three breath odour judges sequentially sniffed mouth air from the panellist, and made an assessment of breath odour and residual flavour present.

Statistical analysis

Statistical treatment of the data is summarized in Table 2. The organoleptic assessments were analysed using a linear mixed model with PROCMIXED in SAS v. 8.2 (SAS Institute Inc., Cary, NC, USA). Flavour, time and judge and their interactions were considered fixed effects, while subjects and 'day of trial' random effects. Time dependencies were modelled using a first order autoregressive covariance structure. Flavour and judge effects were measured with respect to the subject by date error strata, whilst time effects and all interactions with time were measured with respect to the residual error. *Post hoc* comparisons of pairs of products were assessed using Student's *t*-tests.

Table 2 Quest breath freshness panel – data analysis

Experimental
Double-blind experimental protocol
Randomized, balanced carry-over experimental design – 4–6 products
Analysis
Analysed by chartered statistician
Generalized Linear Mixed Model (PROCMIXED in SAS)
Autoregressive order 1 model for repeated measures
Nominal and baseline adjusted scales
Results
Omnibus and time sliced models extracted
Post hoc analysis within time slices using *t*-tests
Cross-trial comparisons using baseline adjusted data

Results

Database of VSC-effective flavour materials and flavours

Examples of data obtained for several flavour ingredients and a series of fully formulated flavours designed to be effective breath-freshening flavours, or comparative 'standard' flavours created without the use of the database, are shown in Table 3. Materials vary significantly in their effectiveness at inhibition of VSC production. The data also show that dental flavours created using the database of effective flavour ingredients can achieve significant inhibition of VSC production, in comparison with standard 'shelf' flavours.

Table 3 Database of *in vitro* inhibition of H_2S production by example flavour materials and whole flavours

Material/'flavour'	*VSC reduction (%)*	*VSCRI*
Cinnamon Supra	40	0.61
Green Tea Base	32	0.48
Parsley Herb	44	0.66
Peppermint American Far W.	46	ND
Spearmint American Far W.	43	ND
'Boudicca'	83	1.02
'Theseus'	74	0.93
'Hearakles'	70	0.88
Typical Shelf Flavours	30–50	0.40–0.60

VSC, volatile sulphur compound; VSCRI, VSC-reduction index.

Efficacy of breath-freshening flavours

Breath-freshening flavours vs *control flavours*

A graph showing the breath-freshening effects over time for a dentifrice containing 1% (w/w) of breath-freshening flavour 'Boudicca' compared with a standard control flavour (approximate dosage 1% w/w) is shown in Table 4. Average breath scores were significantly better at both 90 and 120 min time-points when compared by *t*-test after Mixed Model analysis, and showed significant ($P < 0.10$ and $P < 0.05$, respectively) breath-freshening benefits for the breath-freshening-flavoured product.

Table 4 Comparison of breath-freshening flavour *vs* standard flavour

Time (min)	*Normalized deviation from 0 to 5 baseline malodour score*[a]		
	Standard flavour	*'Boudicca'*	*Significance*
0	0.0	0.0	–
30	−1.69	−1.76	NS, $P > 0.10$
60	−1.42	−1.48	NS, $P > 0.10$
90	−1.07	−1.16	S, $P < 0.10$
120	−0.71	−0.83	S, $P < 0.05$

[a]Baseline scores are equal to the arithmetic mean of organoleptic scores at time zero for each product on each day of assessment.
NS, non-significant; S, significant.

Breath-freshening flavour vs *triclosan- or zinc-containing products*

Data on the effects of breath-freshening flavour 'Theseus' at 1.2% (w/w) in comparison with a standard-flavoured (1.2% w/w) product containing 0.1% (w/w) triclosan are presented in Figure 1. There were significant differences in the mean malodour scores at 30, 60, 90 and 120 min for the 'Theseus' product ($P < 0.01$ in all cases) and the triclosan-containing product ($P < 0.05$ at 30 and 60 min, $P < 0.01$ at 120 min) compared with the unflavoured control. The 'Theseus' product was directionally superior to the triclosan product at all time-points, but the differences were not significant ($P > 0.05$). Similar data comparing breath-freshening flavour 'Herakles' (1.3% w/w) with a standard-flavoured (1.3% w/w) product containing 0.2% (w/w) zinc sulphate are presented in Figure 2. Both the 'Herakles'-flavoured

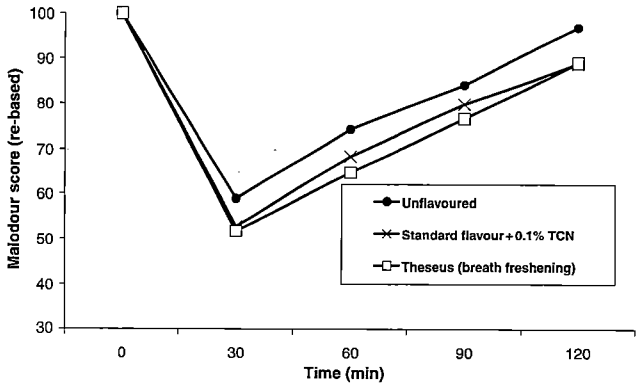

Figure 1 Comparison of efficacy of breath-freshening flavour 'Theseus' *vs* 0.1% triclosan-containing flavoured product and unflavoured control. Y-axis re-based to time zero (pretreatment) = 100% for ease of cross-trial comparison

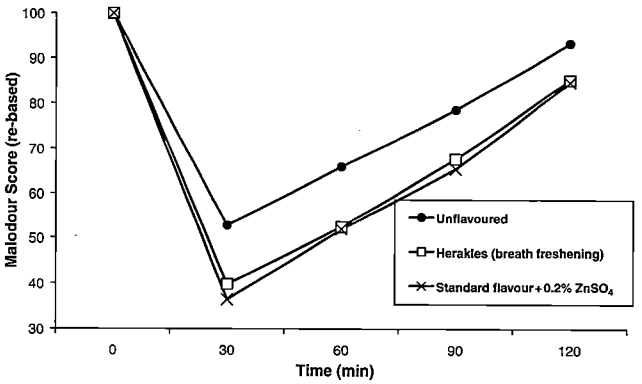

Figure 2 Comparison of efficacy of breath-freshening flavour 'Herakles' *vs* 0.2% zinc sulphate-containing flavoured product and unflavoured control. Y-axis re-based to time zero (pretreatment) = 100% for ease of cross-trial comparison

product ($P < 0.01$ at 30, 60 and 90 min, $P < 0.10$ at 120 min) and the zinc product ($P < 0.05$ at 30, 60 and 90 min) were significantly superior to unflavoured control. There were no significant differences between the breath-freshening-flavoured product and the zinc sulphate product ($P > 0.05$).

Discussion

In the last 3 years, we have carried out a large number of breath-freshening studies, using different product formats and various experimental designs. A combination of the results of these trials, together with those from our *in vitro* assay of flavour efficacy has allowed the development of rules, which allow reliable creation of flavours to deliver breath-freshening benefits. These rules are described in greater detail in the patent application WO2004073668 (Behan *et al*, 2003). The data presented above provide compelling evidence that the Quest breath-freshening flavour rules can allow creation of flavours that deliver significant breath-freshening benefits in consumer products for several hours.

The *in vitro* data presented above provide at least one mode of action for this beneficial effect, as the results show that complete flavours, compounded to optimize the VSC-inhibiting potential, inhibit H_2S production efficiently at relatively low concentrations (500 p.p.m., 0.05% w/w). Because oral care products are normally dosed at between 1.0 and 1.4% (w/w) (10 000–14 000 p.p.m.), the concentrations of flavours in the mouth would be in excess of that required to inhibit VSC production at least initially. We have limited data to suggest similar effects of these ingredients on microbial production of other sulphides (data not shown). It is also possible that these materials could inhibit other bacterial enzymic routes to oral malodorants.

Retention of flavour materials in the buccal cavity is not well understood. We have carried out headspace studies to quantify (in mouth air) the residence times of dental flavour components. These studies have shown that residence times vary significantly across commonly used flavour materials. For example, the half-life of hexanol is of the order of 30 s, whereas the half-life of limonene (the main terpene in orange oils) is more than 20 times longer. An understanding of how these factors influence retention allows optimization of the process of creation to deliver superior benefits from flavours. Further details of these studies are provided in the published patent application WO2004073669 (McNulty *et al*, 2003).

A further aim of the present study was to compare the efficacy of breath-freshening flavours in products with 'true' activities. A number of previous studies have examined the effects of dentifrices supplemented with triclosan (e.g. Gerlach *et al*, 1998; Sharma *et al*, 1999) or zinc salts (e.g. Brunette *et al*, 1998) on organoleptically measured bad breath. The majority of these studies have provided evidence that antimicrobial activities can deliver significant breath-freshening benefits. However, different testing protocols and a variety of malodour scoring scales make extensive comparisons between studies difficult. The present study demonstrates that dental flavours, designed iteratively using both *in vitro* VSC data and results from breath freshness trials, can deliver significant benefits that are comparable with the most commonly used antimicrobial agents in dentifrices.

Other studies (e.g. Rosenberg *et al*, 1992; Waler, 1997) have examined effects of product formats such as mouthrinses or chewing gums. We have carried out trials to examine the effects of these flavours in other product formats, including chewing gum, mouthwash and breath film. Breath-freshening trial results showed once again that the breath-freshening flavours outperformed equivalent standard flavours (data not shown).

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ORIGINAL ARTICLE

The analysis of characteristics of elderly people with high VSC level

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OBJECTIVE: The purpose of this study was to examine the characteristics of elderly subjects who had objectionable levels of volatile sulfur compound (VSC).
SUBJECTS AND METHOD: In 2002, a total of 115 85-year-old persons in Japan were subjected to oral examinations, tongue coat collections, measurements of VSCs levels inside the mouth using a portable gas chromatography (Oral Chroma^{TM}, Abilit, Japan), and assessments of quality of life (QOL) using an SF-36 questionnaire.
RESULTS: Sixty-six of the subjects were edentulous and 49 were dentulous. They were divided into two groups by VSC levels, those with oral malodor (both H_2S > 112 ppb and CH_3SH > 26 ppb; subjects with oral malodor, OM group; n = 7) and those without (n = 108). Our results showed that tongue coat deposit amounts and proportion of dentulous subjects were significantly higher in the OM group. Further, in an analysis of QOL, the SF-36 scores for vitality, social functioning and mental health were significantly higher in OM.
CONCLUSION: We found that elderly subjects with oral malodor tended to be dentulous and had large deposits of tongue coating. However, oral malodor in the OM group subjects did not appear to cause a disadvantage in their social lives.
Oral Diseases (2005) 11 (Suppl. 1), 80–82

Keywords: volatile sulfur compound; oral malodor; elderly; quality of life; SF-36

Introduction

Elderly people generally perceive that oral health is very important to their quality of life (QOL) in a variety of settings, such as eating and communication (McGrath and Bedi, 1999), and typically consider that it will be hindered by the existence of oral malodor. Many studies that have been undertaken regarding oral malodor have used a biomedical or clinical approach, with relationships with periodontal disease (Bosy *et al*, 1994), and with the degree of tongue coating and volatile sulfur compounds (VSCs) values (Miyazaki *et al*, 1995) reported. However, there are few known reports regarding the relationship between oral malodor and QOL that have used a scientific behavioral approach.

In the present study, we tested elderly subjects at 85 years of age. Recently, the so-called 80–20 movement has been promoted in Japan, which advocates that a healthy situation for the whole body exists when a person has a majority of original teeth and aims to have 20 teeth or more remaining when a person is 80 years old. Dental checkups on a nationwide scale were performed for people who were 80 years old in 1997 to prove this speculation and a database was constructed based on the results. We checked the health condition 5 years later of some of the subjects in the 80–20 database.

Subjects and methods

In 2002, a total of 115 elderly people in Japan, each 85 years old and in general good health, were examined, during which we carried out oral examinations including tongue coat deposit collection, measurements of VSC levels inside the mouth using a portable gas chromatography (Oral Chroma^{TM}, Abilit, Japan), and assessments of QOL. From the results, we attempted to determine whether oral malodor in healthy elderly people was related to one of the tested factors or to their QOL. A single examiner evaluated the subjects' tongue coat status and the score from 0 to 3 was given according to the distribution area (Miyazaki *et al*, 1995). Tongue coat samples were collected by scraping the dorsum surface with a sterile tooth brush and suspending the material in PBS. After sonication, the optical density of the suspension was determined at a wavelength of 500 nm. For detailed QOL assessments, we employed the SF-36, Japanese edition version 1.2 (Fukuhara *et al*, 1998a,b), which is a questionnaire used to present general health status as an index for evaluation of QOL. In the SF-36, one item is designed to assess any perceived change in health status and the remaining 36 items are divided into the following eight sections; physical functioning (PF), role-physical (RP), body pain, general health, vitality (VT), social functioning

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(SF), role-emotional, and mental health (MH). Data were analyzed statistically and all statistical analysis procedures were conducted using SPSS 11.5J (SPSS Inc. Chicago, IL, USA).

Results

The average number of teeth present was 4.30 ± 7.11 in all of the subjects, of whom 66 were edentulous and 49 dentate. We divided the subjects into two groups by VSC levels, those with oral malodor (both H_2S > 112 ppb and CH_3SH > 26 ppb, OM group; $n = 7$) and those without oral malodor (normal group, $n = 108$).

Based on the results of a chi-square test, the proportion of dentulous subjects in the OM group was significantly higher than in the normal group, as shown in Table 1. Further, it was shown that the amounts of tongue coat deposits were significantly higher in the OM group (Mann–Whitney's *U*-test) (Figure 1), although there was no significant difference between edentulous and dentulous subjects.

Table 1 Relationship between oral malodor and presence of teeth

	Oral malodor		
Subjects	*OM*	*Normal*	*Total*
Dentulous	6	43	49
Edentulous	1	65	66
Total	7	108	115

Values shown represent the number of subjects. Analysis was carried out using a chi-square test, $\chi^2 = 5.664$, $P = 0.041$.
OM, subjects with oral malodor (both H_2S > 112 ppb and CH_3SH > 26 ppb); normal, subjects without oral malodor.

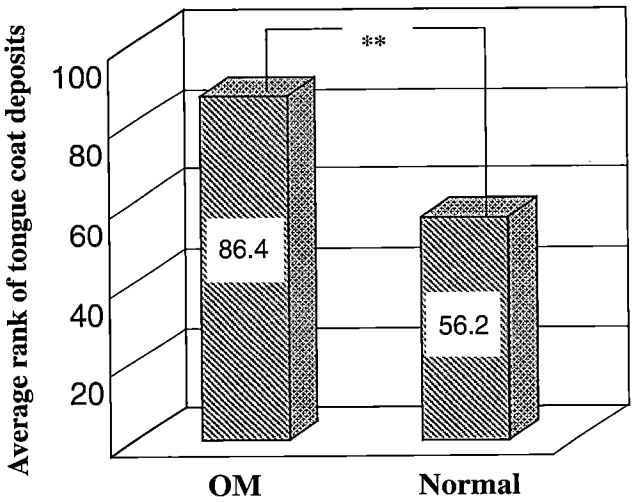

Figure 1 Relationship between tongue coat deposits and oral malodor. Mann–Whitney's *U*-test: $^{**}P < 0.01$. OM, subjects with oral malodor (both H_2S > 112 ppb and CH_3SH > 26 ppb); normal, subjects without oral malodor

Table 2 Factors related to H_2S concentration

	Tongue coat score	*Tongue coat deposit*
H_2S	0.257[a]	0.278[a]

[a]Analysis was carried out using Spearman's correlation coefficient by ranks, $P < 0.01$.

Table 3 SF-36 factors related to CH_3SH concentration

	PF	*RP*	*MH*
CH_3SH	0.187*	0.204*	0.342**

Analysis was carried out using Spearman's correlation coefficient by ranks: $^{*}P < 0.05$; $^{**}P < 0.01$.
PF, physical functioning; RP, roll-physical; MH, mental health.

To examine whether there was a difference in QOL between the OM and normal groups, we analyzed the SF-36 results using a *t*-test and found that scores for the PF, VT, SF, and MH sections were significantly higher in the OM group, which showed that the subjects with oral malodor had a tendency to have high scores for QOL, both physically and mentally. Further, we analyzed which factors were related to the levels of VSC in mouth air, especially the concentrations of H_2S and CH_3SH, and found that the concentration of H_2S was related to tongue coat score and the amount of tongue coat deposit (Spearman's correlation coefficient by ranks) (Table 2). In contrast, CH_3SH was not associated with the amount of tongue coat deposit; however, there were significant relationships to the SF-36 categories of PF, RP, and MH (Table 3).

Discussion

In Japan, more than 50% of dental outpatients are concerned about oral malodor that is actually below the sense of smell threshold and believe that their bad breath, although unable to be detected by others, hinders them from successful interpersonal relationships. In our previous study, we found that healthy high school students who were excessively concerned about bad breath below the sense of smell threshold tended to believe that their interpersonal relationships were not good (Aizawa *et al*, 2003). In contrast, the elderly subjects with oral malodor in the present study did not perceive that their social functioning or mental health were hindered. Further, the concentrations of H_2S and CH_3SH in the mouth air of the present dentulous subjects were higher than in the edentulous subjects.

It is known that the physical functions of elderly people with fewer teeth are higher than dentulous elderly subjects. However, in the present study, the QOL scores of elderly subjects with oral malodor were high both physically and mentally, regardless of the presence of teeth. These results suggest that healthy elderly people who maintained their health condition to the point of being able to come to a health checkup at the age of

85 years old, did not find that bad breath had hindered their social life. We concluded that people who are unable to manage their interpersonal relationships because of oral malodor generally are not healthy at the age of 85 years old.

Based on our findings, we speculate that measurable oral malodor does not cause a hindrance of social and interpersonal relationships, but rather the belief that bad breath exists, which may result in a decrease of QOL. In conclusion, we found that elderly subjects with oral malodor tended to be dentulous and had high levels of tongue coat deposits; however, their social life was not hindered as a result.

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ORIGINAL ARTICLE

Halitosis and periodontal disease in subjects with mental disabilities

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OBJECTIVE: The aim of this study was to investigate the relationship between halitosis, presence of *N*-benzoyl-DL-arginine-2-naphthylamide (BANA) positive activity (indicative of *Treponema denticola*, *Porphyromonas gingivalis*, *Tannerella forsythensis*) and periodontal conditions in subjects with mental disabilities.

MATERIALS AND METHODS: The population consisted of 17 Down syndrome (DS), 17 mentally retarded (MR) and 17 mentally healthy subjects (MH) – control group. A portable sulfide monitor was used to measure the concentration of volatile sulfur compounds (VSC) found in the mouth. Clinical parameters [Plaque Index (PII), Probing Depth (PD), Bleeding on Probing (BOP)] were obtained from six reference teeth. Subgingival plaque samples for BANA Test were taken from the same six teeth and dorsum of the tongue.

RESULTS: PII and BOP were higher in DS than in MR and MH, respectively ($P < 0.05$). Mean PD was similar between DS and healthy groups but it was higher than the MR group. No significant differences were found among the three groups regarding the presence of BANA positive species, however the VSC levels were significantly lower in DS (97.23 ppb) than in MR (203.23 ppb) and MH individuals (180.53 ppb).

CONCLUSIONS: Even though the presence of BANA positive activity was similar among DS, MR and MH subjects, halitosis seemed to be lower in the DS group.
Oral Diseases (2005) 11 (Suppl. 1), 83–85

Keywords: halitosis; Down syndrome; periodontal diseases; sulfur compounds; BANA test

Introduction

Oral production of malodorous substances is most commonly associated with by-products of bacterial metabolic degradation and occurs on oral surfaces, in periodontal pockets and dorsal tongue surface. These products result from microbial fermentation of proteins, peptides, and mucins found in saliva, blood, gingival crevicular fluid, lysed neutrophils, desquamated epithelial cells, and any residual food retained on the oral surfaces. The most conspicuous malodorous compounds are termed volatile sulfur compounds (VSCs). Species that produce such malodorous compounds include *Treponema denticola*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, *P. endodontalis* and *Eubacterium* species (Loesche and Kazor, 2002). Specific microorganisms (*T. denticola*, *P. gingivalis* and/or *T. forsythensis*) from subgingival plaque and tongue-coating samples of individuals with oral malodor have been shown to hydrolyze *N*-benzoyl-DL-arginine-2-naphthylamide (BANA). Since the BANA test detects an arginine hydrolase produced by proteolytic bacteria, this test provides additional information of the microbiota associated with malodor (De Boever and Loesche, 1995; Figueiredo *et al*, 2002).

Halitosis has been correlated with the presence and severity of periodontal disease and by the amount of coating on the tongue. In general, institutionalized individuals with mental disabilities and those with Down syndrome (DS) appear to have an increased susceptibility to periodontal disease. Among DS individuals, the aggressive forms of periodontal disease have been attributed to a possible defect in host defense mechanisms which makes these subjects susceptible to many infections (Izumi *et al*, 1989). This study aimed to evaluate halitosis, presence of BANA positive activity (dental plaque and tongue scrapings) and periodontal parameters in subjects with mental disabilities.

Materials and methods

Study population

The study groups consisted of 17 patients with DS (11 males and six females, 17–35 years of age) and 17 mentally retarded (MR) subjects (10 males and seven females, 18–37 years of age) including individuals with cerebral palsy and birth defects. The population was selected from one institution and as far as possible, the subjects showed similar degree of development disability and parental levels of cooperation. The control group consisted of 17 mentally healthy (MH) subjects with periodontal disease (nine males and eight females,

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17–49 years of age, with at least two sites with attachment level and pocket ≥5 mm). The exclusion criteria included individuals who smoked, had any systemic disease that could influence halitosis, wore dentures, or received periodontal or antibiotic treatments in the previous 6 months. The ethical protocol of this study was approved by the Institutional Committee of Ethics on Human Research.

Clinical examination
After determining oral hygiene condition [Plaque Index (PII)], the supragingival plaque was removed using sterilized gauze. The amount of gingival inflammation was measured by Bleeding on Probing (BOP) and Probing Depth (PD) at six points around each representative tooth (3, 8, 14, 19, 24 and 30).

Volatile sulfur concentration
Volatile sulfur compound measurements were made using a portable industrial sulfur monitor (Halimeter, Insterscan Model RH-17R, Interscan Corp., Chatsworth, CA, USA). Three measurements of the peak (maximum) were taken, and the mean of these values was determined in parts per billion (ppb) sulfide equivalents (Rosenberg *et al*, 1992).

BANA test samples
Subgingival plaque samples were collected from the deepest site presenting BOP in each of the six reference teeth and from the dorsal surface of the tongue, using Gracey curettes. The samples were directly wiped onto a BANA test card and placed in an incubator at 55°C for 15 min. Results were scored as either: blue spots (positive) and no color change (negative).

Statistical analysis
Mann-Whitney *U*-test was used to determine differences among groups in clinical, microbiological and VSC levels. The frequency of plaque accumulation, BOP and BANA positive results were evaluated by chi-square test. The relationship between the VSC and other parameters was obtained by the Pearson correlation. The level of significance was set at 5%.

Results

Fifty-one people (30 males and 21 females; 17–49 years of age) presenting 301 examined sites participated in this study. Table 1 demonstrates that PII, % sites with scores 2–3 and presence of BOP were significantly ($P < 0.01$) higher in DS (% scores PII 2–3 = 100.0; % BOP = 94.8) than in MR (% scores PII 2–3 = 100.0; % BOP = 74.5) and MH (% scores PII 2–3 = 41.2;% BOP = 46.1) subjects, respectively. The PD was similar in DS (3.55 ± 0.9 mm) and in the MH group (3.25 ± 0.8 mm) but higher than in MR (2.65 ± 0.4 mm) subjects ($P < 0.01$). No significant differences were found among the three groups regarding the presence of BANA positive species in subgingival plaque samples. However, the volatile sulfur concentration was significantly lower ($P < 0.01$) in DS (log 1.9 ± 0.2) than in MR (log 2.2 ± 0.3) and healthy individuals (log 2.2 ± 0.2). Considering BANA positive results in tongue, no differences were found among the three groups. Of the control group patients, 10.8% were positive for the presence of BANA activity and the possibility of periodontopathic microorganisms on the dorsal surface of the tongue.

There was no significant correlation between the VSCs levels and the clinical and microbiological parameters.

Discussion

In the present study, the bacterial profiles of subgingival plaque samples and tongue analyzed by BANA test were similar among the three groups of subjects. These results agree with Figueiredo *et al* (2000) and Amano *et al* (2001) studies that showed a similarity between the subgingival microbiota of periodontitis subjects, with and without DS.

The BANA Test is an enzymatic assay and that some species might have more BANA enzyme expression than others. There is a relationship between a BANA positive reaction, bad breath and VSC levels, which emphasis the importance of proteolytic organisms in the process (Figueiredo *et al*, 2002). Likewise

Table 1 Mean (±s.d.) clinical, microbiological and malodor measurements and frequency (%) of Plaque Index, Bleeding on Probing and microbiological parameters

Measurement	*Down syndrome* ($n_{sites} = 97$)	*Mentally retarded* ($n_{sites} = 102$)	*Healthy (control)* ($n_{sites} = 102$)
PII[a,†]	2.75 ± 0.2*	2.36 ± 0.3*	1.76 ± 0.3*
% Sites with PII 2–3[‡]	100.0%	100.0%	41.2%*
Sites BOP[b]+/patient[†]	5.41 ± 0.9*	4.47 ± 0.9*	2.76 ± 1.3*
% Sites BOP+[‡]	94.8%*	74.5%*	46.1%*
Probing depth (mm)[†]	3.55 ± 0.9	2.65 ± 0.4*	3.25 ± 0.8
Sites BANA + (plaque)/patient[†]	4.88 ± 1.2	4.64 ± 1.1	5.05 ± 0.8
% Sites BANA + (plaque)[‡]	85.6%	77.5%	84.3%
% Sites BANA + (tongue)[‡]	7.2%	5.9%	10.8%
VSC[c] (log)[†]	1.94 ± 0.2**	2.20 ± 0.3	2.20 ± 0.2
VSC (ppb)[†]	97.23**	203.23	180.53

[a]Plaque Index (0, 1, 2, 3); [b]bleeding on probing (+ or −); [c]volatile sulfur compounds.
*Significant difference ($P < 0.05$); **significant difference ($P < 0.01$).
[†]Mann–Whitney *U*-test.
[‡]Chi-square test.

we cannot say from the VSC levels which organisms are present.

There are a few species such as *Bacterionema matruchotii*, *Rothia dentocariosa* and *Rothia (Stomatococcus) mucilaginosa* that are known to be BANA positive (C.E. Kazor and W.J. Loesche, unpublished data). These species are not routinely found in subgingival plaques, but could be on the tongue. Some *Capnocytophaga* species are BANA variable *in vitro*, but their BANA activity *in vivo* is not known. They could also be found on the tongue (Kazor *et al*, 2003). Presently it is not known which organisms are giving a positive BANA test in tongue samples.

Although periodontal pockets are believed to be important sites for VSC production, an association between the presence of deep pockets and VSC levels was not established in this and other (Bosy *et al*, 1994) studies. We selected all patients with similar periodontal disease characteristics because of the inclusion criteria of individuals. None of the patients were periodontally healthy.

The VSC levels were lower in the DS group than in MR and MH subjects. This observation suggests that absolute levels of VSC may vary between different population groups. A possible explanation for the low levels of VSC in DS subjects could be related to the role of saliva in malodor. The salivary flow rate in DS individuals has been studied recently (Chaushu *et al*, 2002; Siqueira and Nicolau, 2002) and the findings indicated that salivary flow rate and pH were lower among DS subjects while results for sialic acid, amylase and peroxidase activities showed contradictions. Long-term, a lower saliva flow and a lower pH could result in a microbiota shift to a more acidogenic microbial composition and one would expect this new microbial profile to be less able to produce malodor products (Kleinberg *et al*, 2002). Further studies are necessary to understand the salivary sialometry and sialochemistry for a full understanding of oral physiology and pathology in DS subjects.

In conclusion, even though the presence of BANA positive activity was similar among DS, MR and MH subjects, halitosis seemed to be lower in the DS group.

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ORIGINAL ARTICLE

Subjective patients' opinion and evaluation of halitosis using halimeter and organoleptic scores

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OBJECTIVES: The aim of the study was to evaluate the level of interest in *fetor ex ore* among respondents, patients of the Department of Conservative Dentistry in Warsaw.

MATERIALS AND METHODS: A questionnaire was completed by 295 patients, 202 females and 93 males aged 18–74 years (average 38.73 years). Each person was also examined for organoleptic score and volatile sulfur compounds (VSC) by Halimeter. Halitosis was diagnosed if the average level of VSC was ≥125 ppb and the organoleptic measurement using a 0–5 point scale was ≥2. Statistical analysis was performed using chi-square test.

RESULTS: Incidence of halitosis was greatest in age ranges 25–34 years (29.68%) and 45–54 years (24.52%). Sixty-eight (43.87%) persons with diagnosed halitosis frequently reported having a problem with bad breath and only 5.81% persons with halitosis did not notice this problem ($P < 0.001$). Among 54 healthy persons three (5.56%) reported having a problem with halitosis ($P < 0.001$).

CONCLUSION: A statistically significant correlation was found between clinical organoleptic diagnosis of halitosis and VSC level by halimeter. Subjective patients' opinion correlated well with objective evaluation of halitosis.

Oral Diseases (2005) 11 (Suppl. 1), 86–88

Keywords: halitosis; organoleptic score; volatile sulfur compounds; questionnaire study; Halimeter

Introduction

Halitosis is a condition that has both health and social implications. Current social norms emphasize the importance of personal image and breath malodor may be an important factor in social communication. It is believed that individuals suffering from bad breath are often unaware of it (Rosenberg *et al*, 1995; Rosenberg and McCulloch, 1992). People with elevated volatile sulfur compounds (VSC) levels do not differ from the rest of the population in their level of oral hygiene or number of decayed teeth (Miyazaki *et al*, 1995). The main components of human oral malodor are volatile sulfur compounds, particularly hydrogen sulfide, methyl mercaptan and dimethyl sulfide produced by microorganisms that form tongue coating (Lee *et al*, 2003; Waler, 1997). Concentration of VSC can be measured using a portable sulphide monitor (Rosenberg and McCulloch, 1992).

The aim of the study was to evaluate the level of interest in *fetor ex ore* among respondents, patients of the Department of Conservative Dentistry in Warsaw and their ability of self-assessment of oral malodor level in comparison with objective methods like organoleptic score and VSC concentration measured with a portable hydrogen sulphide monitor.

Methods

A questionnaire was completed by 295 patients, 202 females and 93 males aged 18–74 years (average 38.73 years). Each person was then examined for organoleptic score. VSC values were measured by Halimeter (Interscan Corp., Chatsworth, CA, USA) and recorded using Halisoft software (Ansyco, GmbH, Ostring, Germany). Patients were asked to refrain from eating, drinking or smoking cigarettes for at least 4 h prior to examination and not to brush their teeth the morning before the visit. Halitosis was diagnosed if the average level of VSC was ≥125 ppb (value suggest by software instruction) and the organoleptic measurement using a 0–5 point scale was ≥2. Physiological halitosis was diagnosed if the average level of VSC was ≥75 ppb (value suggest by software instruction) and the organoleptic measurement using a 0–5 point scale was 1. We used a classification of halitosis proposed by Yaegaki and Coil (2000). Statistical analysis was performed using chi-square test.

Results

The incidence of halitosis among patients was greatest in age ranges 25–34 (29.68%) and 45–54 years (24.52%) (Figure 1). People from these age groups are more

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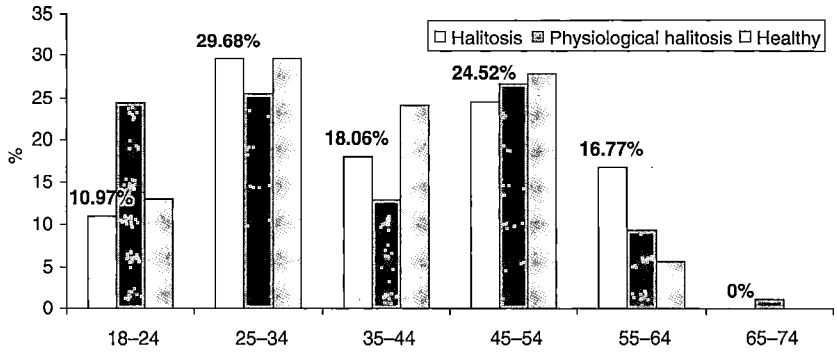

Figure 1 Distribution of respondents by clinical diagnosis and age groups. *Legend*: Halitosis = average level of VSC was ≥125 ppb and the organoleptic score was ≥2. Physiological halitosis = average level of VSC was ≥75 ppb and the organoleptic score = 1. Healthy = average level of VSC was <75 ppb and the organoleptic score = 0. Classification of halitosis proposed by Yaegaki and Coil (2000)

Table 1 Mean organoleptic score and VSC levels by clinical diagnosis

Clinical diagnosis	*Mean organoleptic score ± s.d.*	*Mean VSC ± s.d.*
Halitosis	3.13 ± 0.94	245.9 ± 173.12
Physiological halitosis	1	86.29 ± 20.1
Healthy	0	62.02 ± 11.84

Halitosis = average level of VSC was ≥125 ppb and the organoleptic score was ≥2.
Physiological halitosis = average level of VSC was ≥75 ppb and the organoleptic score = 1.
Healthy = average level of VSC was <75 ppb and the organoleptic score = 0.
Classification of halitosis proposed by Yaegaki and Coil (2000).

Table 2 Self-evaluation of respondent's bad breath

Clinical diagnosis	*Do you have problem with bad breath? Patients' answers**				
	Yes, often	*Yes, sometimes*	*No, rarely*	*No, never*	*Total*
Halitosis	68	70	8	9	155
%	43.87	45.16	5.16	5.81	
Physiological halitosis	4	29	33	20	86
%	4.65	33.72	38.37	23.26	
Healthy	3	18	27	6	54
%	5.56	33.33	50.0	11.11	
Total	75	117	68	35	295
%	25.42	39.66	23.05	11.86	100

*Chi-square test = 118.77; $P < 0.001$.
Halitosis = average level of VSC was ≥125 ppb and the organoleptic score was ≥2.
Physiological halitosis = average level of VSC was ≥75 ppb and the organoleptic score = 1.
Healthy = average level of VSC was <75 ppb and the organoleptic score = 0.
Classification of halitosis proposed by Yaegaki and Coil (2000).

careful about oral heath, so they are more likely to be interested in checking for halitosis. Table 1 shows mean organoleptic score and mean VSC levels for respondents distributed by clinical diagnosis. Among 295 respondents, 25.42% often, 39.66% sometimes, and 34.91% rarely or never had a problem with bad breath. Sixty-eight (46.78%) persons with diagnosed halitosis frequently reported having a problem with bad breath

Table 3 Attention paid by respondents to the bad breath problem between their relatives

Clinical diagnosis	*Have you ever noticed if anybody from your family has problem with bad breath? Patients' answers**		
	Yes	*No*	*Total*
Halitosis	76	79	155
%	49.03	50.97	
Physiological halitosis	37	49	86
%	43.02	56.98	
Healthy	23	31	54
%	42.59	57.41	
Total	136	159	295
%	46.1	53.9	100

*Chi-square test = 1.13; $P = 0.58$.
Halitosis = average level of VSC was ≥125 ppb and the organoleptic score was ≥2.
Physiological halitosis = average level of VSC was ≥75 ppb and the organoleptic score = 1.
Healthy = average level of VSC was <75 ppb and the organoleptic score = 0.
Classification of halitosis as proposed by Yaegaki and Coil (2000).

and only 5.81% persons with halitosis did not notice this problem ($P < 0.001$). Among 54 healthy persons three (5.56%) reported having a problem with halitosis ($P < 0.001$) (Table 2). Clinical tests and treatment procedures allowed diagnosing halitophobia in four cases at the end of the presented study. Respondents noticed a problem with bad breath in their relatives in 46.1%. The frequency of *fetor ex ore* observed in relatives of patients with halitosis was similar to that in relatives of healthy subjects ($P = 0.58$) (Table 3). No correlation was found between cigarette smoking and a diagnosis of halitosis ($P = 0.23$) (Figure 2).

Discussion

There are very few epidemiological studies of halitosis and comparison of the result is rather difficult as the researchers use different criteria. However, many authors reported that in the USA about 50% of the population suffers from halitosis (Rosenberg, 2002). In Japan, among 232 respondents about 47% were sure they had oral malodour, and 51.7% were not until somebody informed them about their diagnosis of halitosis (Miyazaki *et al*, 1995). The conducted study

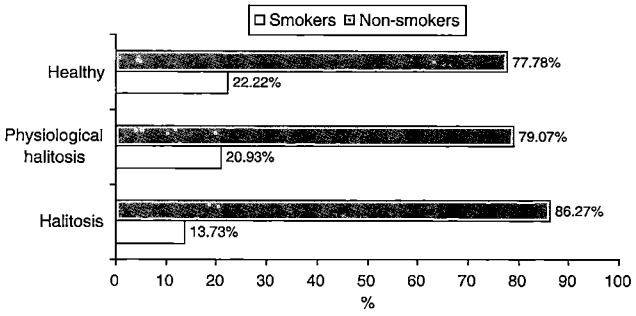

Figure 2 Distribution of respondents by smoking habit and clinical diagnosis. *Legend*: *Chi-square test = 8.08; $P = 0.23$. Halitosis = average level of VSC was ≥125 ppb and the organoleptic score was ≥2. Physiological halitosis = average level of VSC was ≥75 ppb and the organoleptic score = 1. Healthy = average level of VSC was <75 ppb and the organoleptic score = 0. Classification of halitosis proposed by Yaegaki and Coil (2000)

is the first one in a Polish population run on a larger group of patients. It was designed in order to find out whether the problem of halitosis is noticed in our society. The results suggest that a high percentage of the respondents notice a problem with halitosis in themselves or in their relatives. The recognition of halitosis among the respondents correlated well with the results of the clinical examination. The incidence of halitosis in the Polish population is similar to the results presented in the other studies (Miyazaki *et al*, 1995; Rosenberg, 2002).

Conclusions

A statistically significant correlation was found between clinical organoleptic diagnosis of halitosis and VSC level by halimeter. Subjective patients' opinion correlated well with an objective evaluation of halitosis.

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ORIGINAL ARTICLE

Relationship between the SF-36 questionnaire and patient's satisfaction following halitosis therapy

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OBJECTIVE: To assess the health-related quality of life (HRQOL) of patients complaining of halitosis at their first visit and at a later time when their complaint had diminished following therapy, using a self-administered questionnaire, the Medical Outcome Study Short Form-36 (SF-36). The aim of this study was to examine the relationship between HRQOL of patients before and after self-reported disappearance of their complaint following oral hygiene improvements for halitosis.

SUBJECTS AND METHODS: Seventy patients of our special clinic for halitosis served as subjects. At the first visit, each completed the SF-36 before determination of volatile sulfur compound (VSC) concentration in mouth air. After excluding dropouts, the same measurements were performed for subjects whose self-reported complaint had disappeared following oral hygiene therapy.

RESULTS AND DISCUSSION: At the initial visit, SF-36 scale scores for general health, vitality, social functioning, role-emotion, and mental health were significantly lower when compared with the national averages in Japan. For subjects with self-reported disappearance of complaint, only social functioning rose significantly among SF-36 scores at the end of the study. These results suggest that an awareness of improvement in social life could be related to patient's satisfaction with halitosis oral hygiene therapy.

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Keywords: outcome study; Medical Outcome Study Short Form-36; health-related quality of life; halitosis therapy

Introduction

Recently, the patient-oriented outcome of medical intervention has been studied using techniques to evaluate health-related quality of life (HRQOL) with a high degree of reliability. The Medical Outcome Study Short Form-36 (SF-36) health survey is a representative tool for determining HRQOL (Fukuhara *et al*, 1998a) and is widely used in a variety of medical fields. The disappearance of self-reported complaint in halitosis patients is considered to closely depend on HRQOL, however, its determination is not commonly used in halitosis studies. The aim of the present study was to assess the HRQOL of patients complaining of halitosis at the first visit and at the time when their self-reported complaint had disappeared following oral hygiene treatment.

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Subjects and methods

Subjects and therapy

The subjects were 70 patients (20 males, 50 females, mean age 46.7 ± 14.8 years old) who visited the Special Clinic for Halitosis at the Dental Hospital of Iwate Medical University School of Dentistry, Morioka, Japan. None had severe systemic disease and the therapy was not unusual. Briefly, it consisted of instruction regarding oral self-care, professional oral cleaning, and consultations about halitosis including explanation of the examination data, education about halitosis and reassurance. Treatments for periodontal or the other oral diseases were performed if necessary. The SF-36 questionnaire was given and measurement of volatile sulfur compound (VSC) was carried out at the first visit for all subjects, and then for those who no longer complained about halitosis following our therapy.

Definition for disappearance of complaint

For the present study, we defined a patient with disappearance of complaint about halitosis as follows. The patient received treatment three or more times over a 1-month period and, after finishing the therapy, was satisfied that their previous oral malodor was no longer a matter of concern to themselves or others. Further, they agreed to receive regular general dental check-ups after the initial therapeutic period.

Measurement of VSC in mouth air

The VSC levels in mouth air were measured using a Halimeter (Model RH-17K, Interscan Corp., Chatsworth, CA, USA) under the same conditions as reported in our previous study (Kishi *et al*, 2002).

SF-36 questionnaire and statistical analysis

The HRQOL status of each subject was evaluated using the SF-36 version 1.2, Japanese edition (Fukuhara and Suzukamo, 2001), which presents questions related to health status in eight scales: physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotion, and mental health, the answers to which were analyzed using an original algorithm. All procedures concerning the questionnaire were performed according to the manual provided with the SF-36.

For statistical analysis, each of the SF-36 scores was standardized to a norm-based score and shown as a deviation value (mean: 50 ± 10) when compared with the general population in Japan (Fukuhara *et al*, 1998b). VSC concentration results were transferred to logarithms (logVSC) to obtain a near normal distribution. All statistical analysis procedures were conducted using SPSS 11.5J (SPSS Inc., Chicago, IL, USA).

Results

The average Halimeter measurement of all subjects at the first visit was 291.5 ± 417.5, while 64.1% of the subjects had a concentration <200 p.p.b. H_2S equivalent (concentration for the threshold of malodor Tonzetich, 1976). SF-36 scores for the scales of general health, vitality, social functioning, role-emotion, and mental health were significantly lower than the national averages (Figure 1). Table 1 shows the correlations between each SF-36 scale score and two principal components that are commonly considered as physical and mental components (Ware *et al*, 1994). When compared with the standard scores in Japan, it was notable that the social functioning scores of the present subjects had very little relation to a physical component.

During the present study, 17 of the subjects met our definition for disappearance of complaint for halitosis. For them, SF-36 scales and clinical parameters were compared between the first visit and at the end of the study (Figure 2, Table 2). Parameters relating to oral hygiene were significantly improved and mean VSC levels reduced following therapy although the change was not statistically significant. In the SF-36 scores, only the social functioning score rose significantly.

Subsequently, to examine whether the SF-36 is helpful to estimate the prognosis of halitosis patients, we performed a multiple logistic regression analysis, in which SF-36 scale scores for all the subjects were used as independent variables. In addition, the patient residence location information was added to the regression analysis, as the time required commuting between home and clinic might have been a considerable factor for those who dropped out of the study. At the conclusion of the regression analysis, only social functioning and mental health scores were shown to be significant variables, with odds ratios of 0.948 and 1.077, respectively (Table 3).

Table 1 Associations between SF-36 scales and rotated components

	Japanese standard[b]		*Present subjects*	
	Physical[a]	*Mental*[a]	*Physical*[a]	*Mental*[a]
Physical functioning	0.75	0.17	0.75	0.02
Role-physical	0.81	0.19	0.66	0.42
Bodily pain	0.51	0.52	0.62	0.03
General health	0.37	0.66	0.36	0.02
Vitality	0.21	0.88	0.16	0.81
Social functioning	0.45	0.60	0.03	0.70
Role-emotion	0.69	0.34	0.50	0.64
Mental health	0.13	0.89	0.04	0.88

Correlations are shown between each Medical Outcome Study Short Form-36 (SF-36) scale and the principal component.
[a]Two principal (physical and mental) components were extracted from the SF-36 scores of all subjects and rotated to an orthogonal simple structure using a varimax method.
[b]Data from a previous study (Fukuhara *et al*, 1998a).

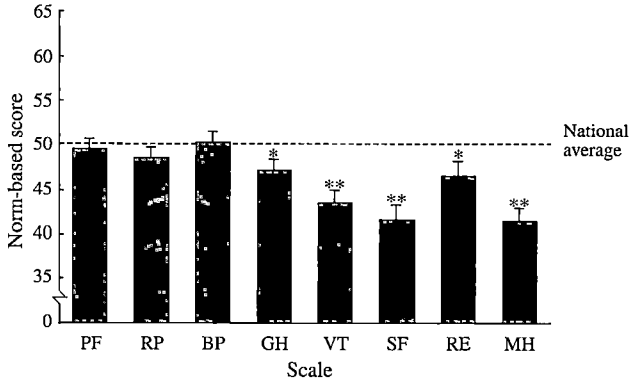

Figure 1 Average Medical Outcome Study Short Form-36 (SF-36) scores for all patients at the first visit. Norm-based score shows mean ± s.d. for the general population in Japan, which is 50 ± 10. The vertical bar (T) shows the s.e. Analysis of each sample was done with a *t*-test by comparing with the national average, $*P < 0.05$ and $**P < 0.01$. PF, physical functioning; RP, role of physical; BP, bodily pain; GH, general health; VT, vitality; SF, social functioning; RE, role of emotion; MH, mental health

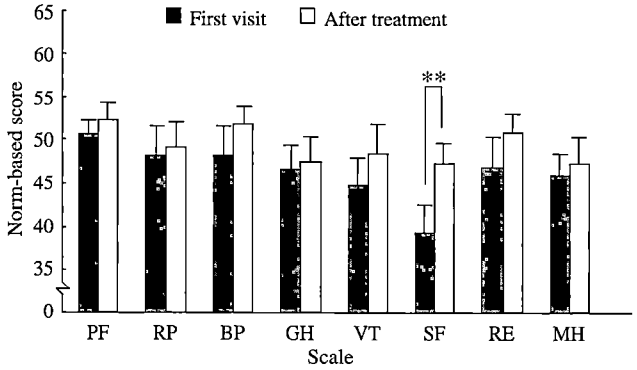

Figure 2 Medical Outcome Study Short Form-36 (SF-36) scores in subjects with disappearance of complaint. Average SF-36 scores at the first visit and following treatment for 17 patients with disappearance of complaint were compared. The vertical bar (T) shows the s.e. **Analysis was done using a paired t-test, $P < 0.01$. PF, physical functioning; RP, role of physical; BP, bodily pain; GH, general health; VT, vitality; SF, social functioning; RE, role of emotion; MH, mental health

Table 2 Clinical parameters before and after the treatment

	First visit	*After treatment*
VSC (p.p.b.)[a]	347.8 ± 91.7	210.8 ± 36.4
logVSC	2.33 ± 0.11	2.23 ± 0.07
Tongue score[b]	1.47 ± 0.24	0.94 ± 0.20**
Pocket depth (mm)[c]	3.33 ± 0.19	3.00 ± 0.22*
Bleeding tooth rate (%)[d]	41.5 ± 5.27	25.2 ± 5.27*
PCR (%)[e]	43.6 ± 5.05	27.1 ± 2.69*

Values represent mean ± s.e.
[a]VSC concentration in mouth air determined by Halimeter.
[b]Tongue coat score obtained by inspection according to a previous study (Miyazaki *et al*, 1995).
[c]Maximum pocket depth among recorded six sites of all measurable teeth.
[d]The rate tooth showing bleeding on probing among all measurable teeth.
[e]Percentage index of Plaque Control Record (Podshadley and Haley, 1968).
Comparison between the averages before and after treatment by paired *t*-test: $*P < 0.05$, $**P < 0.01$.
VSC, volatile sulfur compound; PCR, polymerase chain reaction.

Table 3 Significant variables for prediction whether the complaint could disappear or not

Variable	*B*	*s.e.*	*P*-value	*Exp(B)*	*95% CI*
Social functioning	−0.54	0.24	0.025	0.948	0.904–0.993
Mental health	0.75	0.34	0.029	1.077	1.008–1.152

The dependent variable was disappearance of complaint (disappearance: 1, others: 0). Independent variables were each SF-36 scale score and the subject residence location (same city as our clinic: 1, neighboring city/town: 2, distant city/town with good traffic condition: 3, distant city/town with poor traffic condition: 4, another prefecture: 5). B, partial regression coefficient; CI, confidence interval; Exp(B), exponent of partial regression coefficient (=odds ratio); SF-36, Medical Outcome Study Short Form-36.

Discussion

At the first visit, SF-36 scores for the 70 subjects in the scales of general health, vitality, social functioning, role-emotion, and mental health significantly differed from those of the national standard. Among them, social functioning seemed to best represent the characteristics of the halitosis patients that visited our special clinic. Based on a principal component analysis, social functioning was strongly related to the mental component, however, not the physical component. We considered that this represented a failure in social functioning that was closely associated with the mental health of our patients. These results suggest that, at least for the present subjects, halitosis complaints may be derived mainly from a decline of social functioning.

Among the patients whose complaint was eliminated, average VSC did not show a significant reduction following our treatment. The high standard error of mean VSC values reflects the wide range of VSC levels found in the population at large. This wide scatter may hide the differences obtained through treatment. It is obvious that a reduction of VSC level is the main goal of halitosis therapy; however, in patients whose VSC level is comparatively low at the start of treatment, the outcome of clinical intervention will not be clearly demonstrated. Whilst an improvement in halitosis (as measured by lowering of VSC levels) could not be shown statistically, patients may still show satisfaction following the therapy, as in the present subjects. Our present study suggested that the disappearance of complaint of halitosis in subjects can be related to an improvement in social functioning score following improvement of their oral hygiene.

We tried to develop a prognosis model for halitosis patients by using the SF-36. By means of a stepwise procedure of logistic regression analysis, social functioning and mental health were shown to be significant variables. Furthermore, there was a tendency for patients with low social functioning and high mental health scores to have a high possibility that the initial complaint could disappear by our ordinary remedy for halitosis, although the odds ratios were too small to obtain a sufficient estimation for prognosis.

In conclusion, patient's satisfaction following halitosis therapy relates to HRQOL of the patients, especially to social functioning.

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ORIGINAL ARTICLE

Lethal photosensitization of oral pathogens via red-filtered halogen lamp

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OBJECTIVE OF INVESTIGATION: The ability of laser irradiation in the presence of photosensitizing agent to induce lethal effect on oral bacteria is well documented. We designed an *in-vitro* experiment to achieve phototoxic results on two common oral pathogens, using a high intensity, red filtered halogen lamp. Our goal was to determine the minimum duration of light exposure and drug dilution to achieve at least 50% reduction in bacteria counts.

METHODS: Two common oral pathogens, *Porphyromonas gingivalis* and *Prevotella intermedia* were used in experiments. The source for light energy was a continuous working, high intensity, red filtered, halogen lamp (HL) with light transmitted through a flexible light guide over petri dishes containing live bacteria. Microorganisms were exposed to light for 5, 10 and 20 min. Methylene Blue (MB) in concentrations of 0.1, 0.075, 0.05, 0.025 and 0.01% was used as a photosensitizing agent. Light energy alone and MB alone was used as controls.

RESULTS AND DISCUSSION: Optimum lethal photosensitization (50% or more bacteria killing) of oral pathogens was achieved using halogen light illumination for 5 min and longer with 0.05% MB or exposure to light for 20 min in the presence of 0.025 and 0.01% MB. Light exposure of 20 min in the absence of MB was not effective in killing bacteria. In the absence of light, MB at concentrations of 0.025 and 0.001% was not effective. Reduction of bacteria with the use of 0.05% MB alone was also insignificant. However, 0.075 and 0.1% MB, even in the absence of light was found to be bacteriocidal.

CONCLUSIONS: Our *in-vitro* data indicate that we were able to achieve lethal photosensitization of two common oral pathogens with high intensity red filtered HL in the presence of diluted MB. In this era of increased incidence of antibiotic resistance, bacterial killing with laser or light energy in the presence of photosensitizing agent can prove to be a valuable treatment modality.

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Keywords: photodynamic therapy; photosensitization; periodontopathogens; Methylene Blue

Introduction

The oral cavity of humans is a host place to over 600 of bacterial species (Kazor *et al*, 2003). These bacteria form a unique ecosystem with complex interactions between species with ones producing substrates consumed by others. Some environmental changes, like diet, or decrease of host immune resistance may lead to the overgrowth of unfavorable species, known as periodontopathogens. The most common species included in this category are *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Treponema denticola*, *Actinobacillus actinomycetemcomitans* and *Bacteroides spp*. The named bacteria are associated with chronic periodontitis, which is a major cause of tooth loss in adults when inhabiting the periodontal pockets (Loesche and Kazor, 2002). They are also believed to cause halitosis (or bad breath) when present on the back and base of the tongue (Wilson, 1994; Kazor *et al*, 2003). Traditionally, antibiotics are used to destroy offending bacteria in the cases of inflammatory conditions. Nevertheless, in chronic conditions, such as those caused by the named bacteria, there are many disadvantages in the long-term use of antimicrobial agents, both systemically and topically, notably the development of resistance rendering the agents clinically ineffective and difficulties arising from disturbance of the oral microflora and/or gastrointestinal disturbance. Many therapeutic regimens used for oral infections eliminate both pathogenic and commensal organisms indiscriminately, thereby disrupting the natural ecosystem of the oral cavity (Wilson, 1994).

Photodynamic therapy is a relatively new treatment modality, which was primarily used to destroy tumor cells with the power of laser light energy. Targeting the power to the pathologic cells was reached by staining them with specific dyes that are able to selectively absorb light energy of certain wavelength and are accumulated by tumor cells in lager quantities than by normal cells. The same principle, named lethal

photosensitization, using different dyes (photosensitizers) was later applied to killing different species of bacteria with a significant level of success. Lethal photosensitization is a process by which a photosensitizer is activated by light of an appropriate wavelength resulting in the production of cytotoxic oxygen free radical species, which then kill the target cell (Dougherty *et al*, 1998). Lethal photosensitization is not a specific modality and has been shown to be effective against a variety of cells such as those in neoplasms (Dougherty *et al*, 1990), fungi (Paardekooper *et al*, 1992, 1993, 1995; Wilson and Mia, 1994), viruses (Smetana *et al*, 1998), and bacteria (Bertoloni *et al*, 1990; Dobson and Wilson, 1992; Wilson, 1994; Nitzan *et al*, 1995; Merchat *et al*, 1996; Orenstein *et al*, 1997; Bhatti *et al*, 1998). Bacteria are killed as a result of membrane and DNA damage due mainly to the production of singlet oxygen on irradiation of the dye (Bhatti *et al*, 1998). Work carried out by Dobson and Wilson (1992) using Toluidine Blue O (TBO) as a photosensitizer in combination with HeNe laser light showed that, in dental plaque samples containing *P. gingivalis*, *Fusobacterium nucleatum*, streptococci, black-pigmented anaerobes, and *Actinobacillus actinomycetemcomitans*, all species were susceptible to lethal photosensitization. Furthermore, development of resistance to PDT would appear to be unlikely since its bactericidal activity is due to singlet oxygen and other reactive species such as hydroxyl radicals, which affect a range of cellular targets (Dobson and Wilson, 1992; Paardekooper *et al*, 1992, 1993; Berthiaume *et al*, 1994; Bhatti *et al*, 1998).

The fact that lethal photosensitization is not specific is advantageous in one respect; it is possible to kill all the bacteria present in a mixed infection. However, this also means that commensal bacteria and host tissues could be adversely affected, especially with high-density local power output of the laser light sources. This consideration as well as the others, like hazards related to the use of laser devices, has prevented broad clinical implementation of photodynamic therapy (PDT) in treating different oral conditions.

The goal of this study was to find alternative light source that could be used to apply this promising principle in fighting the unfavorable oral microbiota in broad clinical practice. We assumed that power density produced by high intensity red filtered halogen lamp may be sufficient to activate certain dyes and cause sufficient bacterial killing without serious damage to commensal bacteria or oral mucous lining. This could also eliminate or decrease the other potential hazards associated with the use of laser devises, such as eye damage.

Methods and materials

Bacteria

Prevotella intermedia was isolated from a patient sample material, identified in the laboratory using standard diagnostic test systems (Remel Inc., Lenexa, KS, USA) and maintained by twice-weekly subculture in thioglycollate medium (Becton Dickinson and Co, Sparks, MD, USA). *Porphyromonas gingivalis* ATCC 33277 (Remel Inc.) was maintained by a twice-weekly subculture on CDC anaerobe blood agar (Becton Dickinson and Co.) and in thioglycollate medium (Becton Dickinson and Co.).

Light and photosensitizer

The source for light energy was a continuous working, high intensity 250-W Quartz Halogen light source, model I-250 supplied by Medithon (New York, NY, USA) that is used widely for ENT procedures in the office. The light was transmitted through a flexible light guide and filtered to maintain maximum energy output on the wavelength of 650 nm with broadband red filter (Edmund Optics Inc., Barrington, NJ, USA). The power density measured at 3 cm distance from the end of the light guide with filter was 50 mW cm^{-2}.

A 1% (w/v) methylene blue (MB) solution (Faulding Pharmaceutical Co., Paramus, NJ, USA) was used as a photosensitizer in all experiments. Subsequent dilutions of initial solution (to give 0.1, 0.075, 0.05, 0.025 and 0.01%) were prepared using sterile 10-ml bottles of normal saline and sterile syringes.

Lethal photosensitization of P. intermedia

Petri dishes containing CDC anaerobe blood agar (Becton Dickinson and Co.) were inoculated with 0.5 ml of broth containing 5×10^5 CFU ml^{-1} and left closed at room temperature for about 10 min to let the broth penetrate the agar media. After that plates were exposed to 1 ml of MB solution at concentrations 0.1, 0.075, 0.05, 0.025 and 0.01% for at least 60 s and then exposed to high intensity red filtered light source at 1, 5, 10 and 20 min time intervals. Only plates with exposure to 0.01 and 0.025% MB were used in the experiment with 20 min light exposure. Four plates were used for each experiment. Four inoculated plates that were not exposed to MB or light source were used as controls. In order to examine the ability of light alone to cause bacterial killing, inoculated plates were exposed to the red-filtered light at the same time intervals without previous exposure to MB in the same groups of four plates. To study the ability of MB alone to produce bacterial killing, four inoculated plates were exposed to the MB solution at specified concentrations without subsequent exposure to the red-filtered high intensity light. Samples of the survivors from these procedures were taken from each plate with sterile 10 μl standard loop, dispensed in 1 ml of sterile NS and plated on to fresh media. Plates were incubated in anaerobic conditions in jars using anaerobic pack kits (BBL GasPak Plus; Becton Dickinson and Co.) at 37°C for 24 h and the resulting colony count was performed on each plate.

Lethal photosensitization of P. gingivalis

Serial dilutions of the *P. gingivalis* culture were prepared from the initial culture preserved on plates using sterile 10 μl standard loops (Becton Dickinson and Co.) and sterile NS. The Vitek colorimeter (Hach Company, Loveland, CO, USA) was used to achieve the final concentration of about 5×10^3 CFU ml^{-1} (from an initial

Table 1 Number of survivors (%) following exposure of *P. gingivalis* to different combinations of MB and light

	Light exposure (min)				
MB (%)	*0*	*1*	*5*	*10*	*20*
0 (controls)	75 ± 6	70 ± 6	75 ± 7	73 ± 8	71 ± 8
0.01	70 ± 11	73 ± 6	69 ± 7	55 ± 6	51 ± 4
0.025	75 ± 14	67 ± 4	47 ± 6	48 ± 8	49 ± 5
0.05	68 ± 12	53 ± 10	32 ± 7	32 ± 9	Not attempted[a]
0.075	51 ± 10	45 ± 5	26 ± 5	25 ± 5	Not attempted[a]
0.1	35 ± 9	39 ± 12	28 ± 9	24 ± 4	Not attempted[a]

[a]Exposure of cultures to combination of MB at different concentrations and light for 20 min (last column) was not attempted because significant killing rate was achieved with different combinations of MB and light with shorter time of exposure.

0.5 standard McFarland suspension – 10^8 CFU ml^{-1}). Plates containing CDC anaerobic blood agar (Becton Dickinson and Co.) were inoculated with the resulting suspension using sterile 10 μl standard loops (Becton Dickinson and Co.). The plates were then exposed to the MB solution at different concentrations and thereafter to red-filtered high-intensity light source at different time intervals using the procedure described in the previous experiment. Plates were incubated at 37°C in the tightly closed jars supplied with anaerobic pack kits (BBL GasPak Plus; Becton Dickinson and Co.) for 24 h, and the resulting colony count was performed on each plate.

Statistical analysis
All statistical analyses were carried out using single-factor analysis of variance. Data are mean values ± s.d. of four values (Table 1).

Results

No statistically significant reduction in bacterial counts was noted with the exposure of both cultures to the red-filtered high-intensity light source alone without the exposure to MB. No significant difference was noted between the exposure for 10 and 20 min in both species for all concentrations of MB. MB concentrations of 0.075 and 0.1% alone produced bactericidal effect without light exposure that proved to be statistically significant only for 0.1% concentration in *P. gingivalis*. With light exposure for 1 min both 0.075 and 0.1% concentrations of MB produced statistically significant bacterial colony count reduction for both *P. gingivalis* (Table 1) and *P. intermedius* (similar results; data not presented).

Statistically significant reduction in the bacterial colony count was achieved at concentrations of MB of 0.05% and higher and time of light exposure of 10 and 20 min for both species used in the experiment. Exposure of both cultures to red light produced statistically significant killing at the time intervals of 1, 5 and 10 min with MB concentration of 0.05% and at time intervals of 5 and 10 min with MB concentration of 0.025%. At MB concentration of 0.01% statistically significant killing was achieved only at 10 min light exposure.

Discussion

The results of the study show that exposure of the named bacterial cultures to red-filtered high-intensity light for less than 10 min (with the accumulated energy level of 30 J cm^{-2}) in combination with exposure to MB solution at a concentration of 0.01% and higher produced significant bactericidal effect on both species examined. Significant reduction in bacterial counts can also be achieved with combination of light exposure for 5 min and MB concentration of 0.025% and light exposure for 1 min and MB concentration of 0.05% and higher. MB alone can produce bactericidal effect on *P. gingivalis* at concentration of 0.1%. Exposure to red light with wavelength of 650 nm alone does not produce any significant killing in *P. intermedia* and *P. gingivalis*.

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ORIGINAL ARTICLE

Is transmission of bacteria that cause halitosis from pets to humans possible?

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OBJECTIVE: Bacteria that cause halitosis naturally colonize the animal's mouth. The possibility of transmission of these bacteria from pets to human is an intriguing question. Therefore, the possible correlation between halitosis and regular contact of dental patients with pet animals was the aim of the presented study.

MATERIALS AND METHODS: The study covered 84 patients suffering from halitosis and 40 healthy controls aged 20–62 years. Each person completed a questionnaire and was then examined for organoleptic score, volatile sulphur compounds (VSC) and evaluation of low molecular mass amines in saliva by a ninhydrin colorimetric reaction. Halitosis was diagnosed if the average level of VSC was 125 ppb and the organoleptic measurement using a 0–5 point scale was 2. Statistical analysis was performed using Wilcoxon's and chi-square tests.

RESULTS: The results showed that among the halitosis group over 80% of patients had reported having pets in childhood and over 70% owned a pet at present. In the control group results are 47 and 40%, respectively. Analysis showed a significant correlation between frequency of halitosis and regular contact with pets at present ($P < 0.001$) or in childhood ($P < 0.001$).

CONCLUSION: Pets (dogs, cats) owned in childhood or at present may transmit bacteria that cause halitosis.

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Keywords: halitosis; bacterial transmission; organoleptic score; volatile sulphur compounds

Introduction

Oral malodour affects a large proportion of the population and may be the cause of severe social problems. It has been demonstrated that the tongue coating is the main source of the offensive gases that are responsible for *fetor ex ore*. The major components of oral malodour are produced by gram-negative bacteria for which amino acids are the major source of energy. High protein food is natural for pet animals, dogs and cats. Therefore, bacteria that cause halitosis naturally colonize the animal's mouth. There are diseases for which transmission of pathogens from pets to humans is well proven and not questioned. The possibility of transmission of bacteria responsible for halitosis from pets to human is an intriguing question.

The aim of the study was to evaluate the possible correlation between halitosis and regular contact of dental patients with pet animals.

Methods

The study covered 84 patients suffering from halitosis (mean age 41.2 ± 11.94 years, range from 20 to 62 years; 38.1% males, 61.9% females) and 40 healthy controls (mean age 36.8 ± 11.74 years, range from 22 to 57 years; 27.5% males, 72.5% females). Patients and controls were recruited at the same time. Each person completed a questionnaire and was then examined for organoleptic score and breath volatile sulphur compounds (VSC) by halimeter (Interscan Corporation, Chatsworth, CA, USA). Halitosis was diagnosed if the average level of VSC measured by the halimeter was 125 ppb and the organoleptic measurement using a 0–5 point scale was 2. Because children usually have closer contact with pets than adults, the questionnaire included a question on contact with pets in childhood and adult life. Statistical analysis was performed using Wilcoxon's and chi-square tests.

Results

The results showed that among the halitosis group over 80% of patients had reported having pets in childhood (Figure 1) and over 70% owned a pet at present (Figure 2). In the control group results are accordingly 47 and 40%. Analysis of the results showed a statistically significant correlation between frequency of halitosis and regular contact with pets at present ($P < 0.001$) or in childhood ($P < 0.001$).

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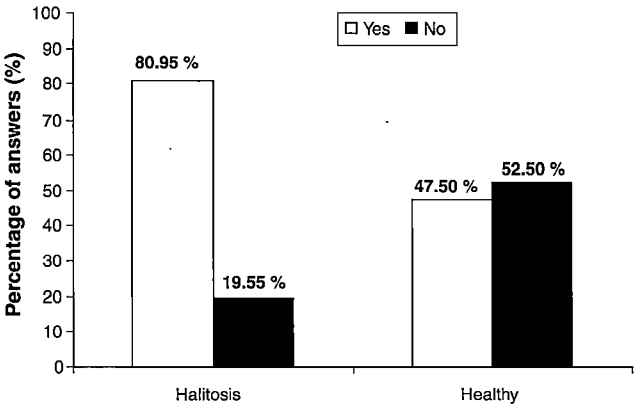

Figure 1 Distribution of patients by clinical diagnosis and having pets in childhood ($P < 0.001$)

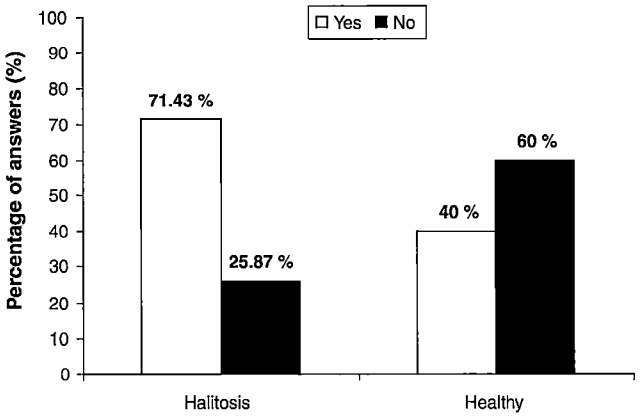

Figure 2 Distribution of patients by clinical diagnosis and currently having pets ($P < 0.001$)

Discussion

In 1960, Keyes had shown that caries in dogs is an infectious disease and transmission of the microbes between dogs is possible (Keyes, 1960). It has been demonstrated in the case of *Streptococcus mutans* that transmission of bacteria between humans is possible as well and is the most common way of spreading the infection (Rogers, 1981; Li and Caufield, 1995). In clinical studies it has been observed that dogs and cats often suffer from halitosis and that the source of odour is plaque deposits on the teeth and tongue of the animals. Species of bacteria found in the mouth of pets suffering from halitosis are similar to those in the human oral cavity in the case of *fetor ex ore* (Simone *et al*, 1997; Culham and Rawlings, 1998; Hennet *et al*, 1998). The present results may suggest that there is a connection between close contact with animals and possible transmission of the bacteria that cause halitosis. However, the present study was carried out on a small number of patients and needs further verification. Transmission of the bacteria may vary according to regional traditions and religious differences around the world. It would also be interesting to investigate if the same genotypes and serotypes of bacterial species are colonizing the mouth of the pet and of the owner.

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Abstracts

Oral Papers

O1
The ADA guidelines on oral malodor products

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This presentation will concern the following: The ADA Acceptance Program, its history and value to ADA members and consumers, procedures for submission of products and role of ADA Council on Scientific Affairs, development of ADA Guidelines for Acceptance of Products, and finally the development of the Guidelines for Products for the Management of Oral Malodor.

O2
Oral malodor research sponsored by industry

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Hill Top Research has been performing oral malodor research for over 30 years, for commercial companies who use these independent clinical studies to support advertising messages. Much of the clinical data generated by Hill Top Research, Inc. does not reach the public domain of peer reviewed scientific journals because client sponsors often consider this research to contain proprietary information. Alternatively academic journals do not consider this type of clinical research to meet sufficiently high basic research standards for publication. This dichotomy results in a knowledge gap between industry and academia as to how malodor research is carried out by industry. The purpose of this presentation is to review the regulatory environments which govern Oral Malodor marketing claims in Europe and North America and thus the clinical research standards which are desirable to substantiate the data to a standard which will withstand competitive legal challenges and regulatory review. The impact of subject data protection laws, good clinical practice guidelines, IRB/IEC review, statistical sample sizes, dental association requirements, and the principles behind sensory research will be discussed in relation to Oral Malodor clinical research. Explanations will be given as to how some requirements may be relaxed depending up whether the purpose of the investigation is 'early stage proof of concept' research evaluating the feasibility of introducing new formulations or ingredients as compared to 'Safety and Efficacy' clinical research carried out on final formulations necessary to meet the requirements of a regulatory submission or challenge in the market place.

O3
Effects of training on experience and non-experience sensory odor judges

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In oral malodor research, sensory odor judging is considered the gold standard to evaluate and rate breath odor quality and intensity. Although training and calibration of sensory odor judges has been well documented in other scientific disciplines (foods/beverages, air/water quality, personal care products), little data have been published on training judges for bad breath assessment.

Objective The purpose of this pilot study was to evaluate the training process, with experienced and novice odor judges, for rating both pleasant and unpleasant odorants.

Methods Six oral malodor sensory judges four with prior experience and two novices were given verbal information on the oral malodor intensity scale (0-5) and were given the opportunity to sniff a reference sample of n-butanol, that had been declared to have a level 3 intensity. Subjects then independently assessed 20 odorant samples for odor intensity (pretest). Four unpleasant and two pleasant odorants, in a variety of intensity levels, and a water sample were included. Subjects then participated in a training program based on the American Society for Testing and Materials (ASTM) standards. Investigators assigned each odorant sample a 'true' intensity score. Subjects' intensity scores were then analyzed as the absolute difference from the 'true' intensity. This dependant variable was analyzed using repeated measures ANOVA.

Results Training significantly ($P = 0.02$) reduced (improved) odor judge error levels, irrespective of previous experience. When comparing improvement between experienced and novice odor judges, there was no significant ($P = 0.99$) difference in the size of deviations from the 'true' scores (errors) between experienced (0.948) and novice (0.950) judges. There was also no difference ($P = 0.94$) in improvement in error levels from pre- to post-test for experienced (pre = 1.13 and post = 0.76) or novice (pre = 1.13 and post = 0.78) judges. There was consistent improvement from pre- to post-test for all odorants ($P = 0.02$; pre = 1.10; post = 0.75) except for dimethylsulfide, which became worse ($P = 0.01$; pre = 0.97; post = 1.58). When comparing the unpleasant *vs* pleasant odorants, there was a significant ($P = 0.006$) difference in error levels (unpleasant 1.09; pleasant 0.70). After removing the water blank scores from the pleasant odorant category the difference was no longer significant ($P = 0.26$).

Conclusions A specific training program for odor judges improved subjects' ability to assess odor intensity irrespective of previous experience. This type of training is recommended prior to conducting oral malodor-related clinical research trials.

O4
An inventory study on a randomized group of 1000 patients visiting a multidisciplinary breath odor clinic at a university hospital

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In 1994 the first European multidisciplinary (periodontology, ENT, psychiatry and occasionally gastro-enterology) outpatient clinic was initiated at the University Hospital in Leuven, Belgium. The first 1000 patient files on the alphabetical list were analysed. It surprisingly consisted of 499 men and 501 women (2–90 years old, mean 38). The majority consulted

spontaneously (69%), others were referred by their house doctor (14%), general dentist (6%) or medical/oral specialists (11%). One-third had complaints since more than 5 years. Two-thirds were informed by confidents or colleagues about their breath odor while they were unaware of it, 15% of the patients noticed it themselves and 18% were informed by others besides noticing it themselves. Not a single patient was alcoholic but 14% were tobacco addicts. In 83.4% of the patients there was an oral problem. Mostly tongue coating or/and periodontitis, was evident. In 3.6% it was a combination of ENT/intra-oral causes. An ENT or gastro-enterologic cause was detected in 3.1 and 1.3% respectively. ENT causes involved postnasal drip, tonsillitis, sinusitis, rhinitis. Only 1% had a metabolic or hormonal problem. The latter regularly also had tongue coating or periodontitis, but the elimination of these causes did not solve the entire problem. On the other hand in 7.6% of the patients no physical cause could be detected, while psychological/psychiatric problems were clearly identified. After the first multidisciplinary examination and treatment initiation/instructions, a control visit was proposed 2–6 months later. All patients were meanwhile referred for follow-up to their general dentist or periodontologist or house doctor/medical specialist. Nearly 60% of the patients did not show up at the second appointment. When a sample was contacted by telephone ($n = 40$) all declared they were cured. 10% of the patients needed 3 appointments or more. From the 31% of the patients who came back for their control visit, 68.5% had experienced a (clear-cut) improvement, and 22.7% noticed a slight to strong improvement. The patients who did not report any improvement (8.8%) were nearly all identified as having psychological or psychiatric traits.

O5

Clinical association of volatile sulfur compounds, Halimeter values, organoleptic score and tongue coating in oral malodor

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Objective Oral malodor is now generally accepted to be the main cause (about 90%) of bad breath. The objective of this study is to evaluate the potential power of volatile sulfur compounds (VSC), Halimeter values, organoleptic score (OLS) and tongue coating in assessing oral malodor.

Methods We investigated the mouth- and nose-breath of 42 subjects with oral malodor, visiting our bad breath clinic. The methods used were gas chromatography, Halimeter measurements, organoleptic scoring (OLS) and tongue coating measurements.

Results The concentrations of hydrogen sulfide (H_2S), methylmercaptan (MM, CH_3SH) and dimethylsulfide (DMS, CH_3SCH_3) in the mouth-breath of the 42 patients with oral malodor amounted to 0.74 ± 0.95 (s.d.), 0.42 ± 0.30, and 0.29 ± 0.19 nmol l^{-1}, respectively. H_2S and MM were absent in nose-breath of all patients, indicating that the origin of these VSC is the mouth. DMS in nose-breath (0.21 ± 0.12 nmol l^{-1}) was similar to that in mouth breath, indicating that the origin of DMS lies outside the mouth. The recognition threshold value of MM is four to 30 times lower than that of H_2S and about three times lower than that of DMS, indicating that MM is the major source of oral malodor. A significant correlation ($r = 0.726$, $P < 0.001$) was found between mouth-H_2S and mouth-MM. No significant correlations were found between these two VSC and DMS. The correlations of H_2S and MM with the Halimeter measurements, with OLS and with tongue coating are all highly significant.

Conclusions Oral malodor is mainly caused by MM. The Halimeter is most sensitive for H_2S. Nevertheless, thanks to the good correlation between H_2S and MM, the Halimeter remains a useful apparatus in predicting oral malodor. OLS and tongue-coating assessments are also useful methods in detecting oral malodor.

O6

The occurrence and cause of extra-oral halitosis

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Objective Oral malodor is now generally accepted to be the main cause (about 90%) of bad breath. Bacterial formation of the volatile sulfur compounds hydrogen sulfide (H_2S) and methylmercaptan (MM, CH_3SH) within the oral cavity is the main cause of oral malodor. The objective was to study the occurrence and cause of the less well-known extra-oral halitosis.

Methods We investigated the mouth- and nose-breath of 59 subjects complaining of bad breath and visiting our bad breath clinic. The methods used were gas chromatography and organoleptic scoring (OLS).

Results Six of 59 patients (10 %) were found to have extra-oral halitosis. The concentrations of H_2S and MM in mouth-breath of patients with extra-oral halitosis were very low and below critical odorous levels. H_2S and MM were absent in nose-breath of all patients. The concentration of dimethylsulfide (DMS, CH_3SCH_3) in mouth- and nose-breath in the six patients with extra-oral halitosis amounted to 1.57 ± 1.31 (s.d.) and 1.64 ± 1.29 nmol l^{-1} (range: 0.50–4.50), respectively, compared to 0.29 ± 0.19 and 0.21 ± 0.12 nmol l^{-1}, respectively, in patients with oral malodor. The critical odorous concentration of DMS is about 0.5 nmol l^{-1}. Simulated DMS gas-mixtures had the same smell as observed in the extra-oral halitosis patients. In these six patients good to perfect correlations were found between the OLS of mouth- and nose-breath (simple correlation coefficient $r = 0.840$, $P < 0.05$), between mouth-OLS and the concentration of DMS in mouth-breath ($r = 0.794$, $P < 0.05$) and in nose-breath ($r = 0.824$, $P < 0.05$) and between nose-OLS and the concentration of DMS in mouth-breath ($r = 0.963$, $P < 0.001$) and in nose-breath ($r = 0.955$, $P < 0.001$). A perfect correlation was obtained between mouth- and nose-DMS ($r = 0.994$, $P < 0.001$), indicating that DMS does not

originate in the mouth . The origin of DMS must be found elsewhere, most probably in the blood.

Conclusions About 5–10% of patients with chronic halitosis has extra-oral halitosis. In all patients with extra-oral halitosis seen thus far, DMS was always the cause of bad breath. Other causes known, such as trimethylamine in the fish-odor syndrome, are very rare.

O7
The treatment of oral malodor

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Objective Because the increase in the bacterial load on the tongue is paramount in the etiology of malodor, we sought to reduce this load by debriding the tongue in combination with short-term usage of antimicrobial mouthrinses.

Methods Subjects with oral malodor had an OR score >2, had whole mouth volatile sulfur (VSCs) levels ≥200 ppb (Halimeter®), and had a positive tongue BANA test. The subjects were given a tongue scraper and a 16 oz bottle of mouthrinse (Listerine® or Ther-a-sol®, single blind design). They were instructed to debride their dorsal tongue surface, to brush and floss the teeth, to rinse with 1 oz of the mouthrinse for 1 min, instructed not to eat or drink for 2–3 h thereafter, and to repeat the protocol in the morning and evening for 7 days. After 1 week all malodor measurements were repeated. The patients were then given a zinc containing tongue gel and instructed to debride the tongue surface, repeating this procedure twice a day.

Results and discussion Thirty-two subjects entered this program, 31 returned 1 week later, and 20 returned for a follow-up examination (average of 37 weeks). The OR score decreased from 2.8 to 0.9 after 1 week of treatment, and to 0.6 for those who returned for the follow-up. The VSCs decreased from 339 ppb prior to treatment to 120 ppb after 1 week and to 53 ppb 37 weeks later. Eighty-one percent of the tongues were BANA positive prior to treatment, 16% after 1 week and 0% after 37 weeks. The microbial flora changed from one dominated by asaccharolytic species to one dominated by saccharolytic species such as *S. salivarius*.

Conclusions This report describes a long-term beneficial effect of treatment on oral malodor.

O8
Impact of periodontal therapy (including antiseptics) on tongue coating and malodour

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Background Bad breath is often caused by periodontitis and/or tongue coating. This study aimed to follow, over a 6 months period, the impact of periodontal therapy on several parameters linked with oral malodour.

Methods In this double-blind, randomized, long-term, parallel study 45 moderate periodontitis patients without obvious tongue coating were enrolled. Besides a 'one-stage, full-mouth' disinfection and oral hygiene improvement (including tongue scraping), patients were instructed to rinse with one of the following products (randomly allocated): CHX 0.2% + alcohol (Corsodyl®), CHX 0.05% + CPC 0.05% and no alcohol (Perio.Aid® Maintenance, a new formulation), or the placebo of the latter for 6 months. At baseline and after 3 and 6 months, a serious of parameters was recorded including VSC rating (Halimeter), tongue coating and an estimation of the microbial load (anterior & posterior part tongue, saliva, supragingival plaque).

Results Even though the initial VSC values were not high (±90, only 18 patients >100), significant ($P < 0.05$) reductions could be recorded in the CHX and CHX+CPC group, but only to a minor extent in the placebo group. Tongue scraping resulted in a significant reduction ($P \leq 0.05$) of the coating up to month 6 in the placebo and CHX+CPC group, but not in the CHX group. The microbial changes in the placebo group were never significant (≤0.3 log values), even though the tongue had been scraped daily. The CHX and CHX-CPC group showed, in comparison to baseline, significant ($P < 0.001$) reductions in the number of anaerobic species in the supra gingival plaque, in the saliva and on the anterior part of the tongue. For the posterior part of the tongue the microbial changes remained ≤0.3 log values.

Conclusions The results of this study indicate that, in patients with moderate periodontitis, periodontal therapy including tongue scraping did not have a significant effect on the microbial load of the tongue or the VSC level, except when combined with a mouthrinse.

O9
Creation of oral care flavours to deliver breath freshening benefits – an *in vitro* method

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Flavours conventionally help to deliver breath freshening in oral care products via sensory masking. The aim of this study was to determine whether flavours could additionally inhibit formation of malodorous compounds by bacteria. Oral malodour is in large part related to the production of volatile sulphur compounds (VSCs) by Gram-negative organisms. We therefore developed a quantitative *in vitro* assay for hydrogen sulphide production by *Klebsiella pneumoniae*. The entire Quest dental flavour palette was screened for inhibition of H_2S production. A database of flavour ingredient effects was created, and used to create VSC-inhibitory flavours. These flavours were assessed in the *in vitro* assay to confirm that the compounded flavour performed as expected. The best performing flavours developed in this way were selected for testing in the Quest Breath Freshness Panel (see poster, P21). Rules based on the performance of dental flavour ingredients, singly or in combination, were also used to develop a patent position. Flavours formulated using these rules (termed Q-fresh flavours) have been incorporated into a range of oral care products including toothpastes, mouthwashes, chewing gums and breath films. These products have been tested in the

Quest Breath freshness panel, and have been shown to deliver significant breath freshening benefits. An *in vitro* VSC-inhibition method has allowed reliable development of flavours with breath-freshening benefits.

O10

The technology behind Colgate® Total™ toothpaste

M Williams, DC ummins
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In the early 1990s, a break-through toothpaste, known as Colgate® Total™, was launched with documented long-lasting activity against plaque, gingivitis, calculus, tooth decay and bad breath. The technology behind this toothpaste is the combination of triclosan, a polyvinyl methyl ether maleic acid (PVM/MA) copolymer and sodium fluoride (TCF). The function of the copolymer is to ensure optimal oral retention and prolonged release of the antibacterial triclosan. Effective levels of triclosan have been measured in the oral cavity 12 h after brushing the teeth. This allows prolonged control of oral bacteria that can lead to the formation of dental plaque and gingivitis and bad breath. Similarly, the retention of triclosan to oral surfaces during 2 years regular use of the product has led to a significant reduction in incremental coronal caries compared to an ADA-approved anti-cavity fluoride toothpaste. Furthermore, significantly less calcium phosphate has been shown to be present in dental plaque after brushing the teeth with the triclosan/copolymer toothpaste, and this has resulted in the reduced formation of tartar. A new variant of the triclosan/copolymer/fluoride toothpaste, having the numerous therapeutic and aesthetic benefits of the original formula, has been made available to consumers. The new dentifrice, which contains an impactful breath freshening flavor, has been documented to be significantly better ($P < 0.05$) than a control toothpaste in providing sustained control of bad breath over 12 h. After 12 h, breath odor was reduced from 51% compared to the control. The long term retention and subsequent release of triclosan by the copolymer in the TCF formula provide consumers protection against plaque, gingivitis, tartar, caries and bad breath.

O11

Effect of oral health care on the recovery from surgery in elderly patients by measuring volatile sulphur compounds

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Oral health care is suggested to be an effective method to decrease pathogenic microorganisms. The purpose of this study was to investigate the effect of oral health care on the recovery from gastrointestinal operations in elderly patients. For this objective, randomized control trials were used for 30 patients (70–80 years old) with gastrointestinal diseases. Infection control was done by antibiotics instillation before and after operations in general and by povidone–iodine solution in the oral cavity both in the intervention (test) group and the control group. For the intervention group, in addition, manual tooth brushing and tongue cleaning were advocated to reduce bacterial putrefaction. A simple gas chromatograph (Oral Chroma®, Abilit Co., Japan) was used to measure hydrogen sulphide, methyl mercaptan and dimethyl sulphide concentrations in the mouth air. Furthermore, oral microorganisms were isolated in tonsils. For 5 days after surgery, the increments of mean values of hydrogen sulphide, methyl mercaptan and dimethyl sulphide in the test group were 0.81 ± 4.86 ng/10 ml, 0.40 ± 1.72 ng/10 ml and 0.16 ± 1.60 ng/10 ml, respectively. On the other hand, those in control group were 2.93 ± 8.09 ng/10 ml, 1.10 ± 2.30 ng/10 ml and 0.36 ± 1.13 ng/10 ml, respectively. The differences in methyl mercaptan concentration between the test and the control groups were statistically significant ($P < 0.001$). In addition, the mean number of kinds of microorganisms in each patient's tonsils in the test and the control groups were 3.08 ± 0.95 and 3.64 ± 1.34 before surgery and were 2.62 ± 0.65 and 3.50 ± 1.74 5 days after, respectively ($P < 0.05$). The oral cavity is a potential reservoir of pathogenic microorganisms. These results demonstrated that oral health care to remove pathogens from dental biofilm or tongue coating can decrease levels of oral malodor and may also reduce the risk of systemic diseases.

O12

Volatile markers of oral malodor in the breath

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Objective To identify the chemical composition of volatile organic compounds (VOCs) in the breath of patients with oral malodor.

Methods Patients with strong offensive oral malodor ($n = 4$) donated oral cavity breath into inflatable bags lined with polyethylene. Patients had grade ≥3 oral malodor on an organoleptic scale (0–5) as assessed by trained organoleptic judges. In the laboratory, each bag was heated to 38°C to vaporize condensed water. 150 ml breath was then removed from the bag and injected on to a sorbent trap (Carbotrap, Supelco) in order to capture the VOCs. Samples were analyzed by automated thermal desorption with gas chromatography and mass spectroscopy (ATD/GC/MS) and the VOCs were identified from a computer-based library of mass spectra.

Results and discussion The 10 most abundant VOCs observed were (in descending order): methylbenzene, 2,2-dimethyldecane, 2,2,3,3-tetramethylbutane, 2-propanone, 3-methyl-5-propylnonane, methylcyclohexane, 3-methylhexane, 2-methyl-1-propene, ethanol and methylcyclopentane. These VOCs may have been produced by bacterial metabolism.

Conclusions Assay with ATD/GC/MS identified the most abundant VOCs in oral cavity breath of patients with strong offensive oral malodor. Potential clinical applications of this technique include identification of the VOCs responsible for oral malodor in individual patients, and monitoring the effectiveness of treatment to reduce the abundance of these VOCs.

O13

Trimethylaminuria, diet and a 'fish-like' odour

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Trimethylaminuria, also known colloquially as the fish malodour syndrome, is a metabolic disorder characterized by the presence of abnormal amounts of the dietary-derived tertiary amine, trimethylamine, in the urine, sweat, expired air and other bodily secretions. Trimethylamine usually undergoes metabolism to form the innocuous metabolite, trimethylamine N-oxide. However, 'greater-than-normal' amounts of trimethylamine may accumulate within the body owing to a mismatch in the enzyme's capacity to undertake this N-oxidation reaction and the substrate load it has to process. Trimethylamine itself is a volatile substance and has the powerful aroma of rotting fish, conferring upon the sufferer a highly objectionable body odour, which can be destructive to the personal, social and work life of the affected individual. As more cases of this condition have been uncovered it has become apparent that there are several subtypes of this disorder, falling into two major categories, and this has allowed a clearer picture to emerge. First, there are those forms that are related to a dysfunction of the normal enzyme (flavin-containing monooxygenase 3; FMO3) activity owing to genetic, hormonal or inhibitory chemical influences. Secondly, there are those forms arising from substrate overload of enzyme activity (either in a normal or depressed state) such as an excess of dietary precursors or variations of gut microflora resulting in enhanced liberation of trimethylamine. Clearly, certain aspects of these two categories are intimately entwined and several factors may act together to give rise to the disorder. In recent years much progress has been made at all levels in our understanding of this condition. This presentation will summarize this progress, draw attention to the different types of the condition and highlight aspects that require further investigation. S.C. Mitchell, R.L. Smith (2001). Trimethylaminuria: the fish malodour syndrome. *Drug Metabolism and Disposition* **29**: 517–521.J.R. Cashman, K. Camp, S.S. Fakharzadeh, P.V. Fennessey, R.N. Hines, O.A. Mamer, S.C. Mitchell, G. Preti, D. Schlenk, R.L. Smith, S.S. Tjoa, D.E. Williams, S. Yannicelli (2003). Biochemical and clinical aspects of the human flavin-containing monooxygenase form 3 (FMO3) related to trimethylaminuria. *Current Drug Metabolism* **4**: 151–170.

O14

In vitro models for oral malodor

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We start with a description of what we mean by a model and introduce the idea of using different types of model to answer different types of questions. A model is a representation of some real phenomena and contains aspects or elements of the real system to be modelled. The model reflects (or duplicates) the type of behaviour (or mechanisms) seen in the real system. The main characteristic of any model is the mapping of elements or parameters found in the system being studied (e.g. tongue dorsum biofilm *in situ*) on to the model being devised (e.g. laboratory perfusion biofilm). Such parameters include correct physico-chemical (abiotic) conditions as well as biotic conditions that occur in both model and reality. The main purpose of a model is to provide information that better explains the processes observed or thought to occur in the real system. Such models can be abstract (mental, conceptual, theoretical, mathematical or computational) or 'physical' e.g. in the form of a real disaggregated *in vitro* system or laboratory model. A wide range of different model systems have been used in oral biofilm research. These will be briefly reviewed with special emphasis on those models that have contributed most to knowledge in breath odour research. The different model systems used in breath odour research will be compared with each other regarding their advantages, disadvantages and main applications. Finally, the requirements for developing an overall 'bad breath model' from considering the processes as a whole (real oral cavity; substrates in saliva;, biotransformation; odour gases in the breath) and extending this to the detection of malodour by the human nose (and all stages in between) will be outlined and discussed.

O15

Use of molecular identification techniques to study oral microbes and microcosms

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Over the last 100 years traditional culturing techniques have shaped our understanding of the oral microflora. However it is generally accepted that around 50% of the oral microbiota is unculturable. Recent advances in molecular biology have given rise to numerous techniques which form the basis of a number of strategies that can be used to detect and identify micro-organisms to levels and specificities unattainable by conventional culture techniques. The molecular biology basis of these techniques can be conveniently split in to two broad groups. (1) DNA hybridization techniques: these are used for slot blot and checkerboard analysis and when combined with microscopy form a powerful visualization technique – fluorescent *in situ* hybridization (FISH). (2) PCR based approach: development of the PCR concept has lead to techniques such as global PCR, specific PCR, nested PCR, PCR-cloning, reverse transcriptase PCR, real time PCR and PCR-DGGE. The above techniques can usually be used on their own however they provide even more power when used in conjunction with each other. For example PCR-cloning or PCR-DGGE can be used to analyse a whole community. From this, un-named or unculturable taxa can be 'identified' and targeted for further characterisation. This may involve the use of PCR specific for the taxon in question, to survey a large number of samples to obtain some prevalence data or in a real time setting to quantify the taxon. Indeed, a fluorescently

labelled probe could be synthesized and used to visualize the taxon in an appropriate environmental sample or microcosm using confocal laser scanning microscopy. All the above techniques add up to a powerful set of tools which if selected appropriately have the potential to revolutionize our understanding of the microbial world.

O16

Organoleptic assessment of pure odour compounds representative of the metabolic end-products of oral microbes

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We have measured various production rates of volatile compounds in tongue scrape cell suspensions, oral biofilm models and the oral cavity *in situ* and wish to relate these to equivalent levels of oral malodour as assessed by the human nose.

Objectives To assess the relationship between measured concentrations of pure odourants and resultant organoleptic scores (using a defined 0–5 score).

Methods Seven odour judges, all with experience of organoleptic assessment, were used in this study for all experiments carried out at a single site (UWE, Bristol). A wide range of pure odourants were prepared at a range of concentrations (from a stock of 0.1 M) and were dispensed as 12 ml volumes into 'universal' bottles (24 ml capacity) leaving a headspace of 12 ml. The bottles were secured with rubber-lined metal caps, labelled with a randomized code and were presented to the odour judges for assessment using the Rosenberg 0–5 scale. The volatile sulphur compounds (H_2S and CH_3SH) were presented as gases with dilutions being prepared through a dynamic gas flow rig (using oxygen free nitrogen as the diluent) and presented to the odour judges at a flow rate of 200 ml min^{-1}. Group means of determinations for each odourant were plotted against log10 of the concentration. Linear regression analysis was used to measure the gradient, 95% CI, and the scatter of the points around the gradient (R^2 value).

Results Organoleptic scores were proportional to the log10 concentration of odourant. Detection thresholds (expressed as mol dm^{-3}) were skatole (7.2×10^{-13}) < methyl mercaptan (1.0×10^{-11}) < trimethylamine (1.8×10^{-11}) < butyrate (2.3×10^{-10}) < hydrogen sulphide (6.4×10^{-10}) < putrescine (9.1×10^{-10}) < dimethyldisulphide (5.9×10^{-8}). Hydrogen sulphide showed highest olfactory power whilst putrescine was the lowest.

Conclusions The olfactory response is exponential in nature. For any single compound, its contribution to malodour depends on: odour (olfactory) power, threshold (of detection) and volatility in addition to the concentration. It is now possible to relate production rates of VC's in models to typical levels of oral malodour perceived by the human nose.

O17

Effect of Probiotic *Streptococcus salivarius* K12 on oral malodour parameters

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Objective Recent non-cultural-based studies of the oral microbiota indicate that some subjects with halitosis are less likely to have the usually predominant commensal bacterium *Streptococcus salivarius* detected [*J Clin Microbiol* (2003) **41**(2): 558–563.]. The aim of this study was to determine whether the oral delivery of viable bacteriocin-producing *S. salivarius* could influence the composition of the oral microbiota implicated in halitosis and impact upon measurable parameters of malodour.

Methods Thirteen subjects with volatile sulphur compound (VSC) breath readings of >200 ppb on two separate visits were recruited for the study. The subjects undertook a 3-day regimen of chlorhexidine rinses followed at intervals by the sucking of four lozenges, each containing $>1 \times 10^9$ colony forming units of the bacteriocin-producing *S. salivarius* K12. The subjects then ceased using the chlorhexidine and took just two lozenges per day for a total of 2 weeks. At each pre-treatment visit and at 1 and 2 weeks after treatment initiation the subjects were tested for VSC and both saliva samples and tongue swabs were subjected to BANA testing, microbial culture and PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. All measurements were taken in the morning prior to the subjects eating, drinking or using any oral care. The ability of the bacteriocins produced by *S. salivarius* K12 to inhibit bacteria implicated in halitosis was tested *in vitro* by deferred antagonism tests using pure cultures and saliva samples.

Results and discussion The VSC levels of eight subjects were significantly lower when tested 1 and 2 weeks after commencing treatment, three subjects had lower readings only after 1 week and two subjects maintained high levels throughout the study. Reduction of BANA activity and changes in the PCR-DGGE profiles occurred in most subjects following treatment. In deferred antagonism studies, *S. salivarius* K12 inhibited some of the bacterial species implicated in halitosis, and significantly inhibited black-pigmented colony types in saliva samples.

Conclusions The replacement of bacteria implicated in halitosis with the bacteriocin-producing commensal bacterium *S. salivarius* K12 appears to provide an alternative therapy for the long-term reduction of halitosis.

O18

Lethal photosensitization of oral pathogens via red-filtered halogen lamp

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Objectives The ability of laser irradiation in the presence of photosensitizing agent to induce lethal effect on oral bacteria is

well documented. We designed an *in vitro* experiment to achieve phototoxic results on two common oral pathogens, using a high intensity, red filtered halogen lamp. Our goal was to determine the minimum duration of light exposure and drug dilution to achieve at least 50% reduction in bacterial counts.

Methods Two common oral pathogens, *Porphyromonas gingivalis* and *Prevotella intermedia* were used in this experiment. The source for light energy was continuous working, high intensity, red filtered, halogen lamp (HL). The light was transmitted through a flexible light guide over petri dishes containing live bacteria. Microorganisms were exposed to halogen light for 5, 10 and 20 min. Methylene Blue (MB) in concentrations of 0.1, 0.075, 0.05, 0.025 and 0.01% was used as a photosensitizing agent. Light energy alone and MB alone was used as controls.

Results and discussion Optimum lethal photosensitization (50% or more bacteria killing) of oral pathogens was achieved under following conditions.1. Halogen light illumination for 5 min and longer with 0.05% MB.2. Exposure to light for 20 min in the presence of 0.025 and 0.01% MB.Light exposure of 20 min in the absence of MB was not effective in killing bacteria. In the absence of light, MB at concentrations of 0.025 and 0.001% was not effective. Reduction of bacteria with the use of 0.05% MB alone was also insignificant. However, 0.075 and 0.1% MB, even in the absence of light was found to be bacteriocidal.

Conclusions Our *in vitro* data indicates that we were able to achieve lethal photosensitization of two common oral pathogens with high intensity red filtered HL in the presence of diluted MB. In this era of increased incidence of antibiotic resistance, bacterial killing with laser or light energy in the presence of photosensitizing agents, can prove to be a valuable treatment modality.

O19
H_2S inhibits O_2^- scavenger and causes oxidative damage *in vivo*

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Objective Volatile sulphur compounds (VSCs), namely hydrogen sulfide and methyl mercaptan have been demonstrated to initiate or progress periodontal disease by increasing the permeability of sulcal epithelium and the degradation of extracellular matrices. Superoxide dismutase (SOD) is one of the most important antioxidative enzymes in intracellular protection against superoxide free radicals which may cause cancer or aging process. The objectives of this study were to demonstrate that hydrogen sulfide, a major contributor to oral malodor, inhibits SOD activity, and to determine if VSCs cause oxidative damage.

Methods Cu,Zn- and Mn-SOD, and human gingival fibroblast (HGF) SOD activities were examined with the determination of the inhibitory activity of the production of 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium formazan. Western blot analysis was employed to determine the effect of hydrogen sulfide on SOD molecules. Oxidative damage was determined with comet, caspase III and other assays.

Results and discussion The activity of Cu,Zn-SOD (0.01 U ml^{-1}) was found to decrease by 83% when exposed to 52 ng/10 ml of hydrogen sulfide for 60 min ($P < 0.0001$ by ANOVA and $P < 0.001$ by Tukey's multiple comparison test) and that of HGF-SOD decreased by 46% ($P < 0.0001$ by ANOVA and $P < 0.001$ by Tukey's multiple comparison test). The inhibition of SOD by H_2S was both time- and concentration-dependent. Following exposure to VSC, SOD activity resumed after incubation in air. The results suggested that the inhibition might be reversible. VSC might cleave the disulfide bonds and might produce monomeric SOD resulting in reversible inhibition. However, Western blot analysis has not demonstrated monomeric SOD. Comet assay and other examination demonstrated that VSC caused oxidative damage and apoptosis.

Conclusion SOD is the body's major defence against oxidative damage. Cu,Zn- and Mn-SOD, and human gingival fibroblast (HGF) SOD activities were strongly inhibited by hydrogen sulfide. The inhibition of SOD resulted in oxidative damage and apoptosis.

O20
Psychophysical aspects of odour

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The history of psychology during the last century should certainly include a statement about the neglect of the crucial topic of human emotion and also ignorance concerning the role of the olfactory sensory system in human behaviour. During my research career, together with my research students, these are topics I addressed. The sense of smell is not a vestigial system but a sensory input of vital importance in human behaviour. Our experiments involved behaviour and psychophysiological and brain activity studies. Some of these studies involved collaboration with members of Quest working in sensory evaluation. Currently it is slowly being recognized that all human sensory input is integrative and each of the sensory systems is important.

O21
Malodour in denture wearers

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A significant proportion of the adult population wear complete or partial dentures, a proportion increasing with age. Denture plaque has not been studied to the same extent as dental plaque, and although there are many similarities in microbial composition, there are some significant differences. Plaque on denture fitting surfaces, particularly of the upper denture, is associated with denture-associated stomatitis, with the aetiological agent generally acknowledged as the yeast *Candida albicans*. In addition, poor denture hygiene and a more acidogenic denture plaque appear to be factors contributing to the condition. Anaerobic microorganisms, although present

in denture plaque, have been specifically investigated rarely. Teeth adjacent to partial dentures are also more susceptible to caries and periodontal diseases, perhaps due to an increased plaque buildup at the prosthesis/tooth interface. Dentures are fabricated from polymethylmethacrylate, using casts constructed from impressions taken in the mouth. The denture-fitting surface is not smooth (although the lingual/labial surfaces are polished) hence plaque accumulates more readily at the relatively stagnant sites. Over time, the epithelial surfaces in the edentulous mouth alter in shape, and dentures may fit less well. Soft liners, or more temporary tissue conditioners are applied to the prosthesis to improve comfort and fit. These materials, are more porous, and may thus entrap and accumulate more plaque biofilm. Little work has been done on malodour associated with denture wearers. There may be a number of reasons for this: (i) the population is deemed to be one which is less cosmetically aware, less likely to invest in products to enhance aesthetics. As the global population ages, and lifespan extends along with enhanced finance, leisure time and health status in selected developed countries, this presumption is perhaps inappropriate.(ii) The nature of malodour in denture wearers is ill defined. The wide age and health range presented by denture wearers poses problems in terms of defining the target populations and specific conditions.(iii) The origin of malodour in denture wearers – and indeed in the elderly in general – is varied: underlying serious illness (malignancy), chronic illness, ulcers, increased medication etc. are all likely to affect breath odour – ammonia, ketones etc.(iv) The increased likelihood of the presence of *Candida* spp. in the mouth may contribute 'yeasty' odours.(v) Poor denture hygiene, accumulation of calculus on prostheses, deterioration and colonization of soft liners will cause significant increases in plaque quantity. Products of deterioration of inert materials in the mouth may also contribute to odour.This presentation will review current status in the area, suggest possible causes of malodour in denture wearers, and consider the need for further work.

O22
Eating behaviour of institutionalized elderly people in relation to tongue cleaning

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Introduction Backing the oral hygiene of elderly people in need of care is an important task for geriatric nursing personal. Tongue cleaning has been generally recommended to improve the oral hygiene status, to prevent the development of oral malodour, and to improve the sense of taste. In Germany, no official recommendations for oral hygiene procedures in hospitals or nursing homes are available and if tongue cleaning is performed a regular toothbrush or a wooden spatula are mainly used. So far, only limited data have been published regarding the influence of tongue cleaning on taste sensation.
Aim Therefore, the aim of this study was to investigate, whether a regime of daily tongue cleaning with a specially designed tongue cleaner could improve the eating behaviour of institutionalised elderly patients.
Methods The study population consisted of 150 edentulous occupants (mean age 74 ± 4 s.d.) of a nursing home in Goettingen. Each subject served as its own control. During the test phase of 2 weeks each participant received five times daily a tongue cleaning procedure using the One Drop Only tongue cleaner (One Drop Only, Berlin; Germany; a combination of a tongue brush and a tongue scraper). During the control run the tongue cleaning was carried out also five times daily over a 2-week period using a regular toothbrush. All procedures were performed by a carefully instructed geriatric nurse. As a measure for the eating behaviour the amount of spurned and returned drinks and food was determined by weighing. The individual impression of taste was also determined by using a questionnaire. All subjects were able to eat and drink without assistance.
Results During the test period the subjects reported an increased appetite and only 18.3% of the served food and 17.1% of the served drinks were spurned and returned. In contrast, during the control phase the subjects returned 52% of the food and 53.2% of the drinks. These differences were statistically significant ($P < 0.05$, t-test n. FERGUSON: $\alpha = 1\%$; $f = 8$; $t = 3.37$).
Conclusion From our data we can conclude that the daily use of a specially designed tongue cleaner (One Drop Only) was capable of improving the appetite of institutionalised elderly patients. (This study was carried out without knowledge and support of the One Drop Only Company).

O23
Predicting bad breath in the non-complaining population

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Relatively few studies have addressed the prevalence and attributes of oral malodor among subjects who do not complain of bad breath. In the present investigation, 88 Israeli adults (mean age 37 ± 9 years) undergoing routine medical checkups, were asked to fill out a questionnaire including 38 questions on general and oral health, dietary habits, and their opinions regarding their own oral malodor levels. Oral malodor assessments included whole mouth odor judge scores on a 0–5 malodor intensity scale, as well as volatile sulfide levels (Halimeter®, Interscan Corp.), and salivary β-galactosidase (OK to Kiss, InnoScent Ltd.). Based on an odor judge cutoff score of 2, the prevalence of bad breath was found to be 29.8 ± 9.6%. Odor judge scores were significantly correlated with both Halimeter® ($r = 0.55$, $P < 0.01$; Pearson) and OK to Kiss ($r = 0.59$, $P < 0.01$; Spearman). Dichotomous analysis of odor judge scores, as compared with detection of salivary β-galactosidase (OK to Kiss) yielded sensitivity, specificity and accuracy of 89, 75 and 79%, respectively. Among the questionnaire results, 10 responses were significantly associated with odor judge scores ($P < 0.05$, unpaired

t-test). When the 10 responses were factored into linear multiple regression analysis to account for odor judge scores, a multiple R of 0.846 was obtained. The results suggest that (i) the prevalence of bad breath in the general population is at least 20%; (ii) the OK to Kiss is a valid test for measuring the presence of oral malodor; and (iii) a simple questionnaire can be used to determine the degree of oral malodor among subjects without a complaint of bad breath.

Poster Papers

P1

Bad breath among young Israeli recruits

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The purpose of the present study was to evaluate the extent of self-reported bad breath in an Israeli population of young army recruits and to assess its relationship with other self-reported parameters, as well as general dental status. Self-reported parameters included smoking status, bad taste, gingival bleeding and the presence of tonsilloliths. The study comprised 426 young adults recruits (ages 18–19), almost all males (95%), all of whom agreed to answer a questionnaire. All participants underwent a dental screening and were divided into three groups regarding to their dental status (do not need treatment, need moderate treatment, need extensive treatment). Statistical analysis included Pearson's chi square test of association using BMDP statistical software. Among the recruits, 142 (33 %) were active smokers. Thirty-five participants (8.2%) reported bad breath as well as bad taste. Twenty-seven (6.3%) reported being told that they had bad breath. Tonsilloliths were reported by 31 participants (7.3%) and gingival bleeding by 80 (18.8%). Self-reported bad breath was positively associated with bad taste, gingival bleeding, the presence of tonsilloliths and general dental status ($P < 0.05$). The study indicates that self reports of bad breath are associated by objective factors (e.g. dental status, tonsilloliths) as well as subjective parameters (bad taste). To our knowledge this is the first report indicating that one young adult in thirteen may suffer from tonsilloliths.

P2

Association between oral malodor and other body odors

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The purpose of the present study was to determine to what extent bad breath is associated with other body odors (axillary odor and pedal odor) in a group of 38 healthy adults. Two odor judges scored malodor from the mouth, tongue dorsum (spoon test), both axillae and shoes of the participants. Volatile sulfides were checked intraorally, as well as by sampling the subjects' shoes. In addition, microbial counts of tongue dorsum (spoon), axillae and shoe insole were assessed using DiaSlides™. In general, significant correlations ($P < 0.05$; Pearson) were found comparing underarm and shoe odor parameters. For example, a correlation of $r = 0.360$ ($P = 0.026$) was obtained comparing left axilla odor score and left shoe odor score of judge 2. Similarly, a correlation of $r = 0.534$ ($P = 0.027$) was found between volatile sulfide levels measured in left shoe, and left axilla odor score of judge 1. In contrast, comparisons of oral malodor parameters did not correlate significantly with the other odors tested. The results do not support the hypothesis that subjects suffering from bad breath have elevated levels of other body odors, as the result of poor personal hygiene habits. Rather, the correlations observed comparing axillary and pedal parameters suggest that these two areas share factors which are not exhibited in the oral cavity, such as similar skin microbiota and perspiration levels, which are not relevant in the case of oral malodor.

P3

The analysis of characteristics of the elderly people with high VSC level

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Objective The purpose of this study is to examine characteristics of the elderly people who had objectionable level of VSC.
Methods In 2002, a total of 115 people (mean age = 85 years) in Japan were surveyed. In the survey, we carried out oral examination, collecting tongue coat, measurement of the volatile sulphur compounds (VSC) levels in mouth air by VSC monitor (Oral Chroma™, Takasago, Japan), and assessment of the QOL using SF-36.
Results and discussion Average number of present teeth among subjects was 4.30 ± 7.11, and edentulous subjects were 66 persons and dentulous subjects were 49 persons. We divided subjects into two groups by VSC levels: the bad breath group (H_2S >112 ppb and CH_3SH >26 ppb) had seven persons, and the group without bad breath had 108 persons. It was shown that the amount of tongue coat was significantly higher in bad breath group ($P = 0.020$), while there was no significant difference in the amount of tongue coat between edentulous subjects and dentulous subjects. And, it was shown that the proportion of dentulous subjects in bad breath group was significantly higher ($\chi^2 = 5.664$; $P = 0.041$). Further, the analysis was performed for the result of SF-36 scores. It was shown that the scores of vitality (VT), social function (SF) and mental health (MH) were significantly higher in bad breath group. Although the levels of QOL had a tendency to be higher in dentulous subjects, there was no significant difference.
Conclusion These results showed that the elderly people with bad breath had tendency to be dentulous and had high deposit of tongue coat. And in this elderly group, people with bad breath tended to have mentally and physically improved.

P4

Parameters related to oral malodor in a population of 71 Israelis

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Bad breath is a common problem which is associated with a variety of factors. The contribution of dental factors related to periodontal health with bad breath remains controversial. Further, because of the problems inherent in odor judge scoring of oral malodor, other adjunct techniques have been proposed. We have recently developed one such colorimetric technique (the 'OK2KISS' test), based on the level of beta-galactosidase in saliva.

Aims The aim of the present study were (i) to determine the factors associated with oral malodour in a group of 71 Israeli subjects, and (ii) to compare existing testing methodologies with a new technique, recently developed in this laboratory (the 'OK2KISS' test).

Methods The study population consisted of a convenience sample 71 Israeli subjects (mean age 36.2 ± 38.4; ages ranging from 15 to 65) who were either undergoing a standard medical (n = 14), checkup, were about to commence orthodontic treatment (n = 12), or were siblings, or parents of orthodontic patients at Tel Aviv University (n = 55). Parameters measured included whole mouth odor judge scoring, halimeter, OK2KISS, gingival index, plaque index and probing depth.

Results Odor judge scores were significantly associated with halimeter (r = 0.55; P < 0.001), as well as the OK2Kiss test (r = 0.52; P < 0.001). However, neither gingival index, plaque index, nor probing depth was significantly associated with odor judge scores or halimeter scores. Logistic regression analysis showed that both halimeter and OK2Kiss scores factored significantly (P = 0.005 and 0.018, respectively, odds ratios 14.9 and 2.7, respectively) in predicting the severity of oral malodour.

Conclusion The study suggests that (i) in this population, periodontal health and oral malodour were not related; and (ii) that the OK2Kiss, together with halimeter scores, can serve to predict the severity of oral malodour levels.

P5

Halitosis and related factors in a Chinese general population

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The purpose of this study was to estimate the distribution of halitosis in a general Chinese population and assess the relationship between halitosis and oral health status and other social or behavioral factors. The subjects were 1000 males and 1000 females aged from 15 to 64 years in urban and rural area of Beijing, China. Questionnaire survey and oral examination was conducted. Questionnaire items included socio-economic status (income and education), oral habits (brushing), knowledge for oral health, dental attendance, life habits (smoking), medical history and self-assessment of halitosis. Oral examination comprised of DMFT, plaque index (PI), calculus index (CI), pocket depth (PD), modified sulcus bleeding index (mSBI) and tongue coating score (TCS). Volatile sulfur compounds (VSC) concentrations in mouth air were assessed by Halimeter. If VSC was 75 ppb and above, the subject was diagnosed halitosis. As a result, the prevalence of halitosis was 35.4% for the total population. Age and area of residence did not related to the VSC value. The levels of VSC were different among the period of assessment time. In the 15–24, 25–34 and 55–64-year-old groups, VSC values were significantly higher in the late afternoon. DMFT was not related with VSC. General health, social and behavioral factors did not relate to VSC. According to the logistic regression analysis, TCS, gender, CI and mSBI was significantly correlated with VSC. TCS had the highest OR value among them. The prevalence of halitosis was high in this population. Tongue coating played the most important role in determining VSC level, followed by periodontal status. However DMFT, smoking, social economic status, oral habits, general health and other social factors did not contribute to the level of VSC. The effective oral health promotion programs would be necessary to improve the poor oral health status and decrease the level of VSC for this study population.

P6

Complaints of halitosis related to the oral cavity. A preliminary report of 700 consecutive patients

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Patients with complaints of halitosis do seek treatment from physicians and dental practitioners, because of the fear that their halitosis may interfere with their social activities. Although the prevalence of halitosis has been reported to be as high as 50%, most physicians and dental practitioners are poorly informed about the causes and treatments of halitosis. In order to care for patients with complaints of halitosis a multidisciplinary team was established at the Erasmus Medical Centre, Rotterdam, The Netherlands. The team included a dental hygienist, an otorhinolaryngologist, and a dentist, who developed a special halitosis programme. One short press release regarding the establishment of the team, was provided to the national press-centre. In the out-patient clinic more than 700 patients were seen by the team. Using a structured questionnaire fed to a PC, patients answered questions regarding complaints about the oral cavity, the upper respiratory tract, the throat, their general health, their cleansing habits of the oral cavity, and prior experiences with general physicians, dental practitioners, and medical specialists. They underwent examinations of the extent of their halitosis, of the perioral and neck region, the oral cavity, the upper respiratory tract, and the upper digestive tract. Finally, the members of the team came to a joint diagnosis and a joint treatment plan for every individual patient. Of the first 700 consecutive patients

around 90% had a natural dentition without removable partial dentures. More than 60% were diagnosed as having periodontal disease with pockets of 4 mm or more in the maxilla. This figure was more than 50% in the mandible. Around 95% had tongue coating.

P7
Complaints of halitosis related to the extraoral cavity. A preliminary report of 700 consecutive patients

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Patients with complaints of halitosis do seek treatment from physicians and dental practitioners, because of the fear that their halitosis may interfere with their social activities. Although the prevalence of halitosis has been reported to be as high as 50%, most physicians and dental practitioners are poorly informed about the causes and treatments of halitosis. In order to care for patients with complaints of halitosis a multidisciplinary team was established at the Erasmus Medical Centre, Rotterdam, The Netherlands. The team included a dental hygienist, an otorhinolaryngologist, and a dentist, who developed a special halitosis programme. One short press release regarding the establishment of the team, was provided to the national press-centre. In the out-patient clinic more than 700 patients were seen by the team. Using a structured questionnaire fed to a PC, patients answered questions regarding complaints about the oral cavity, the upper respiratory tract, the throat, their general health, their cleansing habits of the oral cavity, and prior experiences with general physicians, dental practitioners, and medical specialists. They underwent examinations of the extent of their halitosis, of the perioral and neck region, the oral cavity, the upper respiratory tract, and the upper digestive tract. Finally, the members of the team came to a joint diagnosis and a joint treatment plan for every individual patient. Of the first 700 consecutive patients 57% were women. More than 80% were between 20 and 59 years old. One-third reported that they were never having breakfast or ate only soft food in the morning. Only 2% were diagnosed as having chronic sinusitis, 11% as having pharyngitis, 3% as having laryngitis, and 3% as having tonsillitis.

P8
Halitosis and periodontal disease in subjects with mental disabilities

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Halitosis has been correlated with the concentration of volatile sulfur compounds (VSC) produced in the oral cavity by metabolic activity of bacteria colonizing the periodontal area and the dorsum of the tongue. The aim of this investigation was to study the association among the presence of BANA-positive species (*Treponema denticola, Bacteroides forsythus, Porphyromonas gingivalis*), periodontal condition and halitosis in subjects with mental disabilities. This population (17–49 years of age) consisted of 17 Down Syndrome (DS) patients, 17 with Mentally Retarded (MR), and 17 mentally health (control group). The VSC in the human mouth was determined by a portable sulfide monitor (Halimeter®). Clinical parameters (Plaque Index – PII, Probing Depth – PD and Bleeding on Probing – BOP) were obtained from six reference teeth (# 3, 8, 14, 19, 24, 30). In the same way, subgingival plaque samples were taken from the same selected teeth and from dorsal surface of the tongue and evaluated by BANA™ Test (BANAMet). The results were analyzed by Mann–Whitney *U* Test. PII and BOP were higher in DS patients ($P < 0.01$) and PD was similar in DS and health patients ($P > 0.05$) but higher than MR. No difference was found among three groups for the presence of BANA-positive species, however the VSC levels were lower ($P < 0.01$) in DS (log1.9 ± 0.2) than in MR (log 2.2 ± 0.3) and health (log 2.2 ± 0.2) patients. Although the presence of anaerobic periodontal infection was similar in all groups the VSC levels were lower in Down syndrome patients.

P9
Screening for severe periodontal disease in an elderly population using methyl mercaptan concentration ratio

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Objective The purpose of this study was to examine the usefulness of methyl mercaptan concentration ratio on mass screening for periodontal disease in an elderly population.
Methods One hundred and twenty-two individuals (73 males and 49 females) aged 75 years who had at least 20 teeth in 2003 were chosen from participants of the Niigata Elderly Study (longitudinal study since 1998). Volatile sulfur compounds (VSC) in their mouth such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$) were measured, using portable gas chromatography (Oral Chroma®, Abilit Co Ltd, Osaka, Japan). The subjects were divided into periodontitis/no periodontitis groups based on having at least one site of pocket depth with more than 6 mm (PD 6+ mm). A CH_3SH concentration ratio was defined as $CH_3SH/(CH_3SH + H_2S)$. The receiver operating characteristic curve was plotted to decide the best cut off point of the CH_3SH concentration ratio for screening of subjects with PD 6+ mm.
Results and discussion The best cut-off point of the CH_3SH concentration ratio was 0.35, and the sensitivity and specificity of this cut-off point were 58.7 and 68.4%, respectively. The percentage of subjects with PD 6+ mm in the group of CH_3SH concentration ratio ≥0.35 was 52.9 % (27 of 51) and that in the <0.35 group was 26.8 % (19/71), and, the groups were significantly different ($P = 0.0059$, χ^2 test). It had been reported that VSC, in particular CH_3SH concentration, was significantly higher in persons with periodontal disease than in

periodontally healthy persons. Furthermore, in this study, the use of a portable gas chromatography enabled the VSC measurements for periodontal disease screening outside of the clinic.

Conclusion These findings suggest that CH_3SH concentration ratio may be useful on mass screening for periodontal disease in an elderly population.

P10

Is transmission of bacteria that cause halitosis from pets to humans possible?

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Objective The aim of the study was to determine whether the level of salivary amines in halitosis patients depends on regular contact with pets (dogs and cats) in childhood or at present. It is believed that bacteria metabolizing amine compounds into compounds causing fetor ex ore may be transmitted to humans from animals.

Methods The study covered 84 patients suffering from halitosis and 40 healthy controls aged 20–62 (avg. 39.7). Each person completed a questionnaire and was then examined for organoleptic score, VSC by halimeter and evaluation of low molecular mass amines by a ninhydrine colourimetric reaction. Halitosis was diagnosed if the average level of VSC measured by the halimeter was >125 ppb and the organoleptic measurement using a 5-point scale was >2. Statistical analysis was performed using Wilcoxon's and Chi square tests.

Results Analysis showed a statistically significant correlation between halitosis and regular contact with pets at present ($P < 0.001$) or in childhood ($P < 0.001$).

Conclusions Pets (dogs, cats) owned in childhood or at present may transmit bacteria that cause halitosis.

P11

Correlation of headache and problems of vision with halitosis

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Objective Headaches are a common problem and may occur in response to several factors, e.g. high blood pressure, emotions, changes of atmospheric pressure. It is believed, that amines appearing due to halitosis may take part in this process. Different factors may cause problem of vision among humans. It is presumed that amines detected in halitosis chemically act on the neurological system. This process is sustained and the central nervous system is chemically intoxicated.

Methods Eighty-four patients suffering from halitosis and 40 healthy subjects aged 20–62 (avg. 39.7) participated in this study. Each subject completed a questionnaire and was then examined for organoleptic score, VSC by halimeter and evaluation of low molecular weight amines by ninhydrine colourimetric reaction. Halitosis was diagnosed if the average level of VSC as measured by the halimeter was >125 ppb and the organoleptic measurement using a 5-point scale was >2. Statistical analysis was performed using Wilcoxon's and Chi square test.

Results About 68% of respondents suffering from halitosis had problems with vision ($P = 0.035$). Headaches occurring among halitosis males were rare (20.0%), but were more common in females (43.0%). Differences in the incidence of headaches according to gender and diagnosis of halitosis were not statistically significant ($P = 0.8$).

Conclusion Problems with vision are more common in patients with halitosis. Such problems may be caused by metabolites of low molecular weight amine compounds produced during halitosis, which chemically act on the central nervous system.

P12

Halitometry in patients with chronic caseous tonsilitis prior to CO_2 laser treatment

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Objective Halitosis is a very common complaint at the ENT clinics. The aim of this study is to assess a population with chronic caseous tonsilitis (CCT) and complaint of halitosis prior to criptolisis with CO_2 laser treatment.

Methods Thirty patients were selected for CO_2 laser criptolisis. All patients underwent anamnesis, physical examination and halitometry prior to laser treatment. Halitometry technique followed the *Halimeter Interscan*™ manufacturer's instructions. Results <150 ppb of Volatile Sulphidric Compounds (VSC) were considered normal.

Results Thirty patients, nine male (30%) and 21 female (70%), average age 27.7 years (± 10.4) underwent halitometry. The patients were divided into two groups: Group A – abnormal halitometry (>150 ppb) – five patients (16.66%); Group B – normal halitometry (<150 ppb) – 25 patients (83.33%). Group A results: one male, four female, average age 25.2 (± 5.6). Halitometry was 315.8 ppb (± 164), three patients had caseum at examination (60%). Group B results: eight male, 17 female, average age 28.7 (± 11.2). Halitometry was 47.9 ppb (± 18.6), three patients had caseum at examination (12%).

Discussion Halitosis is a very common complaint among patients with CCT. Only 16% of the patients with CCT and complaint of oral malodor had abnormal halitometry. Nevertheless this population had a high incidence of tonsillary caseum (60%) comparing to a normal halitometry population (12%).

Conclusions Halitometry of VSC is useful to detect halitosis in patients with CCT. The presence of caseum at the examination is a predictor factor of abnormal halitometry. Further studies must be done to assess how CO_2 laser criptolisis could benefit this population.

P13

Withdrawn/transferred/reclassified

P14

Characteristics of a cohort of patients suffering from halitosis and effect of a short-term treatment

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Objectives The aim of this study was to describe the clinical features of a cohort of patients suffering from bad breath observed at the Halitosis Unit of the Department of Stomatology – University of Milano. Moreover, the diagnostic and the short-term therapeutic approach to these patients has been evaluated.

Methods The clinical chart of patients have been reviewed and relevant data collected to be analyzed with an Apple Powerbook G4 PC. Patients complaining of bad breath were admitted to the halitosis unit. At first visit they were submitted to (i) clinical interview (ii) oral and periodontal examination (iii) tongue coating index recording (iv) hedonic breath testing (count-to-twenty test, wrist-lick test, spoon test, floss test) (v) measurement of oral VSC concentration with Interscan Halimeter®. By 1 week patients were submitted to professional prophylaxis and oral hygiene instructions with specific attention to tongue brushing. Patients were then prescribed with no-alcohol chlorexidine 0.2% for 30 s mouthrinse plus 30 s gargle twice a day after brushing. At the control appointment after 2 weeks the tongue coating index, the hedonic tests and the oral VSC measurements were recorded again.

Results One hundred and nine patients (F = 52; M = 59) were observed and treated at the halitosis unit in the period 2000–2002. Thirteen patients were halitophobic without any objective evidence of bad breath whereas 14 had causal ENT diseases. Of the remaining 82 patients with halitosis from oral causes, 60 showed no evidence of active periodontitis. In this latter group a significant reduction of the hedonic test scores and of the oral VSC concentration was found in 85% of patients. Eighty percent also declared a subjective improvement.

Conclusions The clinical protocol described was effective for the diagnosis and treatment of oral halitosis; the treatment with chlorexidine 0.2% was successful in the short-term control of the condition.

Acknowledgement This work was supported by the scientific fund FIRST 2000 – UNIMI.

P15

Non-surgical periodontal therapy reduces VSCs according to disease severity

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The objective of this study was to evaluate the effect of non-surgical periodontal therapy in VSC formation and its relationship with periodontal disease severity. Twenty-five periodontal patients without treatment and a control group with 10 subjects were selected. The patients were classified according to their periodontal diagnosis based on clinical attachment level (CAL): marginal gingivitis (MG), slight periodontitis-CAL = 3 mm (P1), moderate periodontitis-CAL = 5 mm (P2), severe periodontitis-CAL = 7 mm (P3) and terminal periodontitis-CAL > 10 mm (P4). At baseline, all patients received oral hygiene instructions and scaling/root planning. The periodontal parameters: plaque index, gingival index, probing depth and clinical attachment level were evaluated in two experimental periods (T1 = Baseline and T2 = 3 months recall). The Halimeter® was used to detect VSC formation in the same periods. The results after three months showed that VSC formation, probing depth and clinical attachment level decreased similarly in groups MG and P1 ($P < 0.05$). However, P3 and P4 groups showed the highest VSC formation and persistent bleeding in residual periodontal pockets. Within the limits of this study, it could be concluded that non-surgical periodontal treatment may interfere in VSC formation according to periodontal disease severity.

Acknowledgement This work was supported by FAPESP #44052-01.

P16

Effect of intraoral oxygen release device on breath odor

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Objective To assess the effect of intraoral oxygen release device on the reduction of breath odor.

Methods Twenty-two people (age ranged 17–81, mean age = 32, 11 males, 11 females) suffering from breath odor for average 8.3 years across the nation volunteered to participate in this clinical trial. Collection of baseline demographic information and clinical examination was conducted prior to the initial use of the intraoral oxygen release device. After having worn the device at night for 2 weeks, patients were recalled. Concentration of VSC (volatile sulfur compound) using Halimeter® (Interscan Corporation, Chatsworth, CA, USA) was measured and two independent examiners (one male, one female) performed organoleptic measurements, both before and after the use of the device. The two examiners were blinded to the use of device. Data were analyzed with non-parametric Wilcoxon Signed-rank test and proportion using statistical package JMP®5.0.1 (SAS Institute, Cary, NC, USA).

Results and discussion Following use of the device a change in breath odor severity was measured as a decrease of organoleptic breath odor by 58% with one examiner and by 79% with the other examiner. Median VSC level before device use was 111 ppb (parts per billion) and decreased to 75 ppb after device use. The VSC decrease was statistically significant (P-value = 0.004).

Conclusions The study indicated that the intraoral oxygen release device is effective in reducing Volatile Sulfur Compound level and organoleptic breath odor.

P17

Clinical comparison of a triclosan/copolymer/NaF dentifrice and a commercially available breath-freshening dentifrice in reducing breath volatile sulfur compounds overnight: a multiple-use study

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Objective The objective of this randomized, crossover study was to compare the effectiveness of the triclosan/copolymer/NaF dentifrice and a commercially-available breath freshening dentifrice containing fluoride for their ability to reduce volatile sulfur compounds (VSC) responsible for breath odor overnight.
Methods Following a one-week washout period of brushing with a regular fluoride dentifrice, subjects reported to the clinical site without performing oral hygiene, eating or drinking in preparation for baseline breath sampling. Subjects were randomly assigned a test dentifrice and instructed to brush their teeth for 1 min, twice a day for 1 week. On the morning of the 8th day, subjects returned to the test site, having refrained from oral hygiene, eating and drinking, for overnight sampling. Following a second one-week washout period, subjects repeated the same regimen, but now using the other test product. At each measurement, the level of breath VSC was evaluated using a gas chromatograph equipped with a flame photometric detector. Measurements were taken in duplicate, and then averaged. The levels of VSC were expressed as parts per billion (ppb) in mouth air.
Results At baseline, the mean breath VSC levels for the TCF and breath freshening dentifrice groups were 618 and 581 ppb, respectively. There was no statistically significant difference between the groups. Overnight, the TCF and the breath freshening dentifrice reduced breath mean VSC levels to 267 and 521 ppb, respectively. This gave a 56.7 and 10.2% reduction in VSC levels for these two products, respectively, compared to baseline. The reduction for the TCF dentifrice was significantly different ($P < 0.05\%$) from that of the breath freshening dentifrice.
Conclusion The results of this randomized, double-blind, crossover study indicate that the TCF dentifrice was significantly more effective than a commercially-available breath freshening dentifrice containing fluoride in reducing breath VSC responsible for bad breath overnight.

P18

Clinical effectiveness of a triclosan/copolymer/sodium fluoride dentifrice in controlling oral malodor: a three-week clinical trial

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Objective The purpose of the study was to compare the effectiveness of a dentifrice containing 0.3% triclosan/2% polyvinylmethylether/maleic acid (PVM/MA) copolymer/0.243% sodium fluoride (TCF) to a commercially-available dentifrice containing 0.243% sodium fluoride (control) for the management of oral malodor in a 3-week, randomized double-blind, longitudinal clinical trial.
Methods A panel of four expert judges used a nine-point hedonic scale to evaluate breath odor using a protocol designed in accordance with the ADA Draft Acceptance Program Guideline for Products Used in the Management of Oral Malodor. Following a baseline evaluation, prospective subjects with hedonic scores above the threshold value for unpleasant breath were stratified by score and randomized into two treatment groups. Subjects brushed their teeth for 1 min with their assigned dentifrice, twice a day, for 3 weeks. Before oral malodor evaluations, the subjects refrained from eating odorigenic foods, from using mouthrinses and breath mints and from performing dental hygiene procedures.
Results Eighty-one adult male and female subjects completed the study. The baseline hedonic scores for the TCF and control dentifrices were 7.80 and 7.84, respectively, corresponding to unpleasant breath. The final mean oral malodor scores for the TCF dentifrice differed significantly from the baseline and control values ($P < 0.05$) for every time point examined (1.5-, 4.0-, 12.0-h and 1-, 2- and 3-week intervals). The mean final breath scores for the TCF dentifrice group were 3.06, 3.48, 3.42, 3.66, 3.42 and 3.36, respectively, for each time point. These scores correspond to pleasant breath. Conversely, the control dentifrice group scored at levels either above 5.0 (before 12 h) or above 7.0 (after 12 h) which corresponded to neutral or unpleasant breath.
Conclusion In conclusion, the results of this double-blind clinical study clearly indicate that a dentifrice containing triclosan/copolymer/NaF provides effective control of oral malodor for up to 12 h.

P19

A study for the effect of tongue cleaning

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The objective of this study was to evaluate breath odor reduction after giving tongue cleaning instruction to adults. The breath odor of 26 subjects (ages 19–77) was measured before and after tongue cleaning instruction. They were instructed to scrape away tongue coating with a Dental Pro multi-tufted tongue brush (Jacks co. Ltd.) once or twice a day. They used the tongue brush until they could see no more plaque on their tongue. Oral malodor was evaluated using: two portable sulphide monitors (Halimeter RH-17, Interscan Co.) and Brethtron, (Yoshida Co.), gas chromatography (GC-8A, Shimazu) and organoleptic measurements. Organoleptic measurements were estimated on a scale of 0 to 5 as follows : 0 = no odor, 1 = barely noticeable odor, 2 = slight odor, 3 = moderate odor, 4 = strong odor,

5 = severe odor. The subjects were asked to refrain from brushing, eating, drinking, smoking and using mouthwash 2 h before each examination. Tongue coating status was evaluated in five grades by inspection, according to distribution area and thickness of tongue coating. Probing depth, bleeding on probing and dental plaque were assessed. One week of tongue cleaning resulted in reductions in tongue coating status and breath odor. As a result of the before and after measurements, no correlation was observed between tongue cleaning as instructed and bleeding on probing or dental plaque. According to a questionnaire given 1 week after the study, tongue coating status had reverted to pre-cleaning condition in about 1 day. Therefore, we concluded that tongue cleaning should be done at least once a day to control breath odor.

P20
Effect of protease tablet on reduction of tongue coating

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Objective Practical treatment of halitosis requires tongue cleaning since volatile sulphur compounds (VSC) seems mainly to be from the tongue coating. From this point of view, mechanical tools such as tongue brushes or scrapers have been developed. However, approaches by chemical tongue cleaning have not been reported. Thus we developed tablets containing protease from kiwifruits, which could resolve tongue coating, and assessed the effects of the protease tablet to control tongue coating.

Methods Crossover studies and double blind experiments were designed using volunteers with informed consent. The trial was done twice per volunteer, that is, they had a tablet with or without the addition of protease from kiwifruits (test and placebo) with intervening washout periods of at least 2 weeks. The degree of change in tongue coating was evaluated visually using a tongue coating score which consisted of an area component (0–3) and a thickness component (0–3). An image analyzer was also used to measure the changing in actual area of coating.

Results The average value of the tongue coating scores after taking a test tablet (11.4 ± 5.2) was significantly smaller ($P < 0.01$) than before taking the tablet (18.8 ± 7.0). Image analyzer measurements also showed significant reduction ($P < 0.01$) of tongue coating by taking test tablet. On the other hand, a placebo tablet showed no significant effects in both analyses.

Conclusions This study indicated that taking protease tablets could reduce tongue coating. We are planning further clinical trials that can show reduced VSC concentrations in mouth air with decreasing tongue coating.

P21
Design and operation of a human breath freshness panel

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Breath freshening products are the fastest growing sector in the oral care marketplace. There is a clear need for robust testing protocols to evaluate the relative effectiveness of these products. The objective of this study was to design and set-up an organoleptically-assessed human breath freshening panel to allow reliable and robust comparative assessment of a range of oral care products. Initially, 10 prospective breath-odour judges were assessed in a Quest standard test for skills in recognition, differentiation, ranking and descriptive assessment of chemical odorants. The four highest scoring judges were further trained in assessment of chemical malodours relevant to breath freshening, using odorants including methyl mercaptan, indole, skatole, cadaverine, putrescine, butyric and isovaleric acids. An extensive programme of sniffing the breath odour of volunteers was undertaken, scoring breath using both the 'Rosenberg' (0–5) intensity scale, as well as a residual flavour (hedonic/pleasantness) scale. Comparative scores were analysed to ensure reasonably low inter-judge variability. A panel of members of the public were recruited as the Quest Breath Freshness panel. Panellists were assessed by a qualified dentist, and those with severe tooth decay, extensive dentures or severe periodontal disease were excluded, as were diabetics, pregnant women or those with any pre-disposition to respiratory illness. The panel has been used to conduct breath assessments prior to product use, and at a range of set time-points thereafter, up to 4 h. Results are routinely analysed by ANOVA. Trials were further optimized to use a 4- or 5-week, crossover, double-blind experimental design. Data from the panel has been used to support product claims, and to assess the efficacy of combinations of flavours, antimicrobial agents and oral product formats in delivering breath freshening benefits. The panel now runs routinely, and is used for research and commercial assessment of product benefits. The Quest breath freshness panel provides robust experimental data on the effectiveness of oral care products for breath freshening.

P22
Clinical efficacy of a triclosan/copolymer/NaF dentifrice and a commercially available breath-freshening dentifrice on hydrogen sulfide-forming bacteria

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Objective The objective of this double blind clinical study was to compare the 4-h and overnight effects of a triclosan/copolymer/fluoride (TCF) dentifrice and a commercially available breath freshening (CBF) dentifrice containing fluoride for their ability to control H_2S-forming bacteria.

Methods Following a one-week washout period of brushing with a regular fluoride dentifrice, subjects reported to the clinical site without performing oral hygiene, eating or

drinking in preparation for baseline saliva sampling. After providing a baseline saliva sample, subjects were randomly assigned a test dentifrice and instructed to brush their teeth for one minute, twice a day for one week. On the morning of the 8th day, subjects returned to the test site having refrained from oral hygiene, eating and drinking for overnight sampling. Subjects then brushed for 1 min with the assigned dentifrice, and returned for 2- and 4-h postbrushing evaluations. Following a second one-week washout, subjects repeated the same regimen, but now using the other dentifrice. Oral microflora samples were collected by subjects rinsing with 10 ml of sterile water for 10 s. Each collected sample was serially diluted and plated in duplicate onto lead acetate agar. After incubating for 72 h, dark colonies were counted, expressed as log colony-forming units ml^{-1}, and reductions from baseline were calculated.

Results Compared to baseline, the TCF dentifrice reduced H_2S-forming bacteria 0.82, 0.80 and 0.22 log units at the 2-, 4- and 12-h time points compared to 0.60, 0.43 and 0.07 log units for the CBF dentifrice. The TCF dentifrice was statistically significantly better ($P < 0.05$) than the CBF dentifrice at 4 h and overnight after brushing the teeth.

Conclusion These results support data obtained in organoleptic and gas chromatography studies and strongly suggest that the TCF dentifrice is effective in controlling bacteria implicated in the formation of bad breath.

P23

Discrimination of oral-halitosis substance under humidity-rich environment by using QCM sensor array and a preconcentrator

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Objectives A halitosis test for objective evaluation is required. Halitosis is due to Volatile Sulfur Compounds (VSCs) produced by bacterial metabolism. In this study, we proposed a method for discriminating among sulfur compounds using a Quartz Crystal Microbalance (QCM) sensor array combined with a preconcentrator. The adjustment of the temperature profile, the optimization of the flow path sequence and the use of a dehumidification filter enabled VSC measurement under a high-humidity condition. Moreover, the result of principal component analysis indicated that three types of VSCs were separated using the sensor-array output pattern.

Methods Multiple QCMs with different coating films were used as a sensor array and the pattern recognition of eight different sensor responses enabled the odor identification. The preconcentrator was used not only for improving limit of detection but for eliminating the influence of humidity by adjusting the desorption temperature. The headspace over the liquid (water or ODO) where VSC was dissolved with the concentration of 0.1% (w/w) was used as the samples.

Results and conclusion An experiment on the discrimination among VSCs was performed. The headspace vapor of each sample was supplied to the preconcentrator for 30 s, followed by an exposure of the vapor thermally desorbed at 170°C to the QCM sensor array. The sensing films suitable for VSCs were selected among the various candidates and the peaks of the sensor responses to VSCs were analyzed using principal component analysis. Each sample was measured 10 times at the intervals of 30 min. As a result, it was found that three kinds of VSCs were successfully discriminated. Furthermore, it was found that the sensor responses increased in case of longer accumulation time. Thus, it is possible to detect the sample with lower concentration. This measurement method will be effective to diagnose halitosis objectively.

P24

Comparison of sampling bags used for measuring breath odor

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The use of sampling bags to collect breath odor has not been well established. The aim of this study was to compare and judge the quality and performance of three commercially available sampling bags. An aluminized polyethylene bag (Aluminum Bag, Tokyo Deodorant Inc., Japan), the Tedlar® bag (Tedlar® Bag, Tokyo Deodorant Inc.), and a polyester bag (Flek-Sampler® Nioibukuro; Omi Odorair Service Co. Ltd., Japan) were assessed. A GC instrument (GC14-B, Shimadzu, Japan) equipped with a flame photometric detection system were used to measure the concentrations of volatile sulfur compounds (VSC: hydrogen sulfide, methyl mercaptan, and dimethyl sulfide) released from the sampling bags over time. An electronic nose (e-nose) (Odor Discrimination Analyzer FF-1, Shimadzu, Japan) was used to assess the characteristics of the gas eluted from the inner surfaces of the bags. The GC results showed that the Tedlar® bag had the highest performance, with 73% of the VSC concentration lasting for up to 4 days. The disadvantage of this bag, however, is that the Tedlar® bag itself has an odor that disturbs the e-nose's response and organoleptic evaluation. The polyester bags were only able to hold hydrogen sulfide for 2 h. The e-nose's responses were strongly associated with changes in humidity within the polyester bag. The humidity was more stable within the aluminized polyethylene bag, which was able to sustain 94% of the VSC concentration for up to 24 h. In conclusion, Tedlar® bags are beneficial for GC usage. Aluminum coated polyester bags are beneficial for GC measurements, e-nose measurements, and organoleptic judgements. Polyester bags have poor hold of VSC, but the low cost suits required disposability for the measurements of individual patients. Thus, breath odor sampling bags should be selected based on the specificity of the measuring instrument.

P25

Clinical assessment of oral malodor intensity expressed as absolute value using electronic nose

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We have previously reported that the score as determined under top-note mode with an electronic nose provides objective halitosis-related measurements, but those obtained by the multiple linear regression method (EM-MLR) show only relative and not absolute values. The purpose of this investigation was to assess clinically oral malodor intensity expressed as an absolute value using an electronic nose. Sixty-six patients who complained of oral malodor participated. Malodor intensity (EN-MI) calculated by totaling the contributions based on the measurement of multiple category gases and EM-MLR were measured by analyzing mouth air samples with an electronic nose system (FF-1, Shimadzu). Oral malodor was also assessed by an organoleptic test and volatile sulfur compound levels (VSC) using gas chromatography. Probing depth (PD), tongue coating score (TCS), and plaque control record (PCR) were used as oral health parameters. Receiver operating characteristics (ROC) procedures were applied to analyze the diagnostic accuracy of EM-MI using an organoleptic test as a gold standard. The area under the ROC plots for EN-MI (0.975) was significantly larger than that for logVSC (0.896) ($P = 0.036$), but did not differ significantly from that for EN-MLR (0.932). In logistic regression analyses of the possibility of subjects being in the upper 25th percentile of EN-MI distribution as a dependent variable, percentage of teeth with PD.4 mm, TCS, and PCR were each significantly associated with EN-MI and respective odds ratios were 13.0, 7.1, and 28.2. These results suggest that oral malodor intensity expressed as an absolute value using the electronic nose may be useful for evaluating clinically oral malodor.

P26

Breath odor evaluation by detection of volatile sulfur compounds – correlation with organoleptic odor ratings

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Detection of oral volatile sulfur compounds (VSCs) by gas chromatography (GC) is a widely used method for evaluating breath odor. Air aspirated from the mouth is injected into the GC column for analysis. To eliminate discrepancies caused by variations in operator sampling or injection techniques, a new GC system designed to aspirate breath samples directly into the GC was developed.

Objective A clinical study was performed to compare this new automated breath-sampling GC system to organoleptic evaluation by two-trained odor judges.

Methods A randomized, two-cell, double-blind, parallel design was used in which subjects were tested before and 3 h after using either a mouthrinse containing zinc or a matching placebo rinse. Thirteen subjects used the zinc mouthrinse, and twelve used the placebo. Subjects with a wide range of VSC levels were studied. The average organoleptic ratings for each subject at each time point were compared to the average VSC measurements made with the GC, and Pearson product–moment correlation coefficients between the corresponding GC and organoleptic measurements were determined.

Results The correlation between the GC and organoleptic assessment methods were highly significant ($P \leq 0.001$) for: total VSCs, 0.65; H_2S, 0.63; CH_3SH, 0.61; and $(CH_3)_2S$, 0.46. The correlation between the two judges was also highly significant (0.823, $P < 0.001$).

Conclusion These results demonstrate the utility of the automated gas chromatography method for evaluating breath odor.

P27

Comparison of ninhydrine methods of detecting amine compounds with other methods of detection in halitosis

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Objectives Availability of required substrate – amino acids is a factor determining the appearance of halitosis. The aim of the study was to determine whether a chemical test for low molecular weight amines in the saliva may be a new method of diagnosing halitosis.

Methods Eighty-four patients suffering from halitosis and 40 healthy volunteers aged 20–62 (avg. 39.7) participated in the study. Halitosis was diagnosed if the average level of VSC measured by a halimeter was >125 ppb and the organoleptic measurement using a 5-point scale was >2. In all subjects low molecular weight amines were evaluated by the ninhydrine method. Patients with halitosis were randomized into a treatment groups. Zinc tablets, tablets and mouthwash containing chlorhexidine or lyophilized lactic acid-forming bacteria were used. Test subjects were examined at day 0, 7, 14, 21 and after 3 months and controls on day 0, 21 and after 3 months. Statistical analysis was performed using Wilcoxon's test.

Results Analysis showed, that the level of amines was highest in subjects with halitosis (0.39, s.d. 0.06) and correlated significantly with results of VSC measurement and organoleptic scores ($P < 0.001$). Reduction of amine levels after treatment was statistically significant (0.36, s.d. 0.06, $P < 0.001$). The amine levels in healthy controls was lower (0.29, s.d. 0.07) and remained at a similar, stable level.

Conclusions The ninhydrin method of detecting salivary amines may be one of the methods of diagnosing halitosis, because salivary amine levels significantly correlated with VSC levels measured by halimeter and organoleptic scores. This method may also be used to evaluate treatment efficacy.

P28
Analysis of 'Morning Breath'

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The objective of the study was to identify the volatiles responsible for the smell of oral malodour, the so called 'Morning Breath' or halitosis. Therefore the breath of volunteers was trapped on different SPME fibers and on Carbotrap 300 and analysed by Thermodesorption-GC-MS and Thermodesorption-GC-Sniffing Detection. So numerous odour active compounds were identified and their influence on malodour was determined. The identified substances belong to different chemical classes (S-compounds, N-compounds, alcohols, acids, phenols). An *in vitro* aromagram of the malodour was generated. The results indicate that the frequently mentioned 'Volatile Sulphur Compounds' (VSC) H_2S, methane thiol and dimethyl sulfide only play a minor role in the 'Morning Breath'. More important are other sulfur compounds, phenolic compounds (phenol, p-cresol) and N-compounds (indole, scatole).

P29
The effects of essential oils on periodontopathic bacteria and oral halitosis

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Oral halitosis has been associated with periodontal disease. Hydrogen sulphide, methyl mercaptan and dimethyl sulphide are the major components of halitosis originating in the oral cavity. These compounds are mainly produced by periodontopathic bacteria in the oral cavity. In the absence of the bacteria, the odoriferous compounds are not generated. We have demonstrated the antibacterial effects of essential oils on the periodontopathic bacteria such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum*. As essential oils, manuka oil, tea tree oil, eucalyptus oil, lavandula oil, and romarinus oil were used, and their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The essential oils inhibited the growth of the bacteria tested, and manuka oil did so most effectively. MBC values showed that lavandula oil acted bacteriostatically, and the remaining oils bactericidally. Bacterial strains tested were killed completely by exposure for 30 s to 0.2% manuka oil, tea tree oil or eucalyptus oil. This study showed that among the essential oils tested, tea tree oil and manuka oil, particularly the latter, had strong antibacterial activity against periodontal disease associated bacteria. In the present study, we investigated the clinical effects of essential oils in the treatment of oral halitosis. When the degree of halitosis before and after the treatment was evaluated, the essential oils demonstrated efficacy in reducing the level of the three major components of halitosis. From the data of the present study, we consider that essential oils can be used in oral health management.
This study was supported by grant from Kanpou Science Foundation.

P30
Relationship of oral malodor with tongue microbiota analyzed with real-time PCR

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Oral malodor is thought to mainly originate from the bacterial population present on the surfaces of tongue. However, the relationship between oral malodor and tongue microbiota has been little studied. In the present study, we analyzed tongue microbiota with real-time PCR and examined an association of oral malodor with tongue microbiota. The subject population consisted of 37 patients who complained of oral malodor. Oral malodor was assessed by an organoleptic test (OLT) and volatile sulfur compound (VSC) levels by gas chromatography. Real-time PCR was carried out in tongue biofilm samples using a LightCycler^{TM} system as previously described (Kuboniwa *et al.* 2002) and the bacterial proportion in clinical sample was used as a quantitative parameter. The correlation coefficient between the proportion of total five Gram-negative anaerobes (*P. gingivalis*, *T. forsythensis*, *P. intermedia*, *P. nigrescens*, and *T. denticola*) and total VSC level (r = 0.84) was higher than that between the bacterial proportion and OLT score (r = 0.24). In these five Gram-negative anaerobes, the proportions of *P. intermedia*, *P. nigrescens* and *T. denticola* were correlated with the concentration of hydrogen sulfide (r = 0.79, 0.73 and 0.50, respectively). The proportions of *P. gingivalis*, *P. intermedia* and *P. nigrescens* were also correlated with the concentration of methyl mercaptan (r = 0.91, 0.40 and 0.71, respectively). When a linear regression analysis was conducted using the proportion of each of five Gram-negative anaerobes as independent variable, the explanatory power of these independent variables showed 77% for total VSC level and 10% for OLT score. These results suggest that Gram-negative anaerobes in tongue microbiota may contribute VSC production and may be also related to oral malodor.

P31
The relationship between the relative amount of *Porphyromonas gingivalis* in saliva and halitosis

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Objectives *Porphyromonas gingivalis* is one of the microorganisms that most actively produce CH_3SH and we have reported that subjects with *P. gingivalis* have higher CH_3SH levels than subjects without *P. gingivalis*. However, little is known about the relationship between *P. gingivalis* levels in saliva and the condition of oral malodor. In this study, we evaluated the association between the relative amount of *P. gingivalis* in saliva and halitosis in mouth air.
Methods All of the subjects were patients at the Preventive Dentistry and Breath Odor Clinic of Kyushu Dental College, where they received a periodontal examination. Volatile

sulfur compounds (VSC: hydrogen sulfide and methyl mercaptan) were measured using gas chromatography. Saliva samples were collected in a sterile plastic tube over a period of 5 min while the subject chewed on paraffin wax, and were then immediately stored at –80°C until use. Template DNA was obtained from the stored saliva using an Easy-DNA Kit (Invitrogen, CA, USA) according to the manufacturer's instructions. Conventional PCR assays were used to confirm the presence of *P. gingivalis*. A 5' nuclease TaqMan PCR was used to quantify *P. gingivalis* in saliva. The relative numbers of bacteria were measured using the comparative threshold cycle method.

Results We found a quantitative relationship between the *P. gingivalis* levels in saliva and the condition of halitosis in mouth air.

Conclusion We analyzed the relationship between the relative amount of *P. gingivalis* in saliva and oral malodor.

P32

Saliva incubation as replacement for intra-oral malodour evaluation

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Background Breath odor is scored by different techniques, each with its own shortcomings. Organoleptic ratings are uncomfortable for the patient, subjective, influenced by external parameters including food and cosmetics, and lack of international calibration. An intra-oral VSC rating (Halimeter) or gas chromatography can overcome the embarrassing situation for the patient, but not every clinician has this equipment. This project evaluated the reliability of saliva incubation as an indirect, *in vitro*, test for bad breath.

Methods In a double-blind, randomized, long-term, parallel study 45 moderate periodontitis patients that received a 'one-stage, full-mouth' disinfection, oral hygiene instruction (including tongue scraping) and antiseptics. The intra-oral VSC ratings (at baseline, and at 3 and 6 months) were compared to *in vitro* VSC recordings of the headspace air from saliva (0.5 ml, 37°C, 1 and 2 h, anaerobic chamber). The latter recordings were further correlated to the number of CFU in the saliva. At the same time the headspace also received an organoleptic rating.

Results Even though the VSC values remained within a narrow range (90% observation 10–200), a strong correlation was found between the intra-oral VSC ratings and the 1 h ($r = 0.48$, $P < 0.0001$) and 2 h ($r = 0.43$, $P < 0.0001$) VSC production of saliva. The VSC values of incubated saliva correlated strongly ($r = 0.71$) with the CFU for anaerobic species in the saliva. The VSC values and organoleptic ratings of the incubated saliva also correlated strongly ($r = 0.64$ after 1 h and 0.73 after 2 h incubation).

Conclusions Saliva incubation can be used as an indirect way to score bad breath. Due to its simplicity it can be useful in longitudinal studies as alternative for direct organoleptic recordings.

P33

In vitro perfusion biofilm model for the growth of oral microbes associated with oral malodour

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Objectives Oral malodour is mainly due to the generation of volatile sulphur compounds (VSC) by oral anaerobes present in the tongue biofilm. The aim of this study was to evaluate the use of a biofilm system to model the ecology and VSC physiology of tongue biofilm microcosms. Of particular interest was the use of the model to maintain a high diversity of bacterial groups and study their response to environmental changes including exposure to VSC substrates and inhibitors.

Methods Biofilms were grown using a Sorbarod perfusion model modified by supply of anaerobic gas to grow oral anaerobes. Sorbarods were inoculated using tongue scrape suspension containing 3×10^8 cfu ml^{-1} and pre-incubated anaerobically for 24 h at 37°C to promote cell adhesion. After 24 h, the biofilm system was perfused at 12 ml h^{-1} with BHI (0.74 g L^{-1}) supplemented with haemin, cysteine and/or DTT. Biofilms and eluates (released cells) were sampled up to 96 h and numbers of broad bacterial groups enumerated by selective media. VSC production was measured using a Halimeter^{TM}.

Results Repeated analysis of biofilms showed that a quasi-steady state was achieved between 48 and 96 h. The mean specific growth rate was 0.018 h^{-1}. Ecological analysis showed all broad groups were maintained at a constant proportion reflecting the original inoculum for both biofilms and eluates. VSC specific activity was upregulated when biofilms were perfused with appropriate VSC-substrates (cysteine, glutathione and methionine). *In situ* exposure of the biofilms to pulses of putative inhibitors (Zn, H_2O_2 and chlorhexidine) at a range of concentrations typically used *in vivo* gave dose-dependent inhibition of VSC production (for Zn and H_2O_2) and reduced viable numbers by one to two log-fold (H_2O_2 and chlorhexidine).

Conclusions The modified Sorbarod perfusion model is suitable for maintaining high diversity biofilms representative of the tongue flora in terms of both ecological behaviour and response towards VSC substrates and inhibitors.

P34

Effect of a triclosan/PVM/MA copolymer/fluoride dentifrice on volatile sulfur compounds *in vitro*

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Objective The objective of the investigation was to document the *in vitro* efficacy of a triclosan/PVM/MA copolymer/fluoride (TCF) dentifrice against the formation of volatile sulfur compounds (VSC) as well as the growth of H_2S-producing bacteria. Clinical studies using organoleptic judges, gas chromatography, or a portable sulfide monitor have generally been employed in the assessment of treatments for

the control of oral malodor. However, these studies are not appropriate for screening purposes because of the expense and time required.

Methods An *in vitro* method was developed for the purpose of screening new technologies for their ability to control VSC formation and for determining bio-equivalence of efficacy when implementing changes in existing formulations. The method combines basic microbiological methods, dynamic flow cell techniques and head space analysis. The *in vitro* VSC method was validated by comparing the efficacy of two dentifrices containing TCF with a control fluoride dentifrice as the TCF products have been clinically proven to control oral malodor.

Results In the validation studies, the TCF-containing dentifrices were significantly better ($P < 0.05$) than the control dentifrice in inhibiting VSC formation and reducing H_2S-producing bacteria. For example, when compared to baseline, the TCF dentifrices reduced VSC formation between 42 and 49% compared to the control dentifrice which reduced VSC formation 3%. There was no significant difference ($P > 0.05$) between the two TCF dentifrices.

Conclusion Using an *in vitro* breath VSC model, it has been demonstrated that two variants of a dentifrice containing triclosan, PVM/MA copolymer and fluoride have efficacy that is significantly better than a control fluoridated dentifrice and that there is no significant difference between the triclosan/PVM/MA copolymer/fluoride dentifrice variants.

P35

Withdrawn/Transferred/Reclassified

P36

Use of n-butanol as an odourant to standardize the organoleptic scale of breath odour judges

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The alcohol n-butanol has been recommended for use as a standard odorant by various groups for the training or standardization of breath odor judges and sensory evaluation panels.

Objective To assess the use of n-butanol as a suitable odorant for use in organoleptic training of breath judges.

Methods One judge with full smell acuity was trained in the method of organoleptic assessment using odorant solutions of all chemical classes (acids, amines, indole, sulphides) with the exception of alcohols. The subject was proficient in scoring odorant solutions, standard gas mixtures and human breath using the Rosenberg 0–5 organoleptic scale. The judge had gained over 5 years experience of assessment and for the purposes of this work was considered to be the gold standard. A wide range of n-butanol solutions were prepared from 0 up to 90 000 ppm and dispensed as replicate 12 ml volumes in Universal bottles (24 ml) leaving a headspace of 12 ml. Sets of odorants were prepared, labelled by code, randomised and presented to the judge in a completely blind fashion. The judge scored each concentration. This process was repeated on 32 occasions over a period of 12 weeks. Means of data for each determination for each concentration series were plotted against the log concentrations of odorant. Linear regression slope analysis was used to measure slope, the 95% CI of slope and the scatter of points (R^2 value). Head space concentrations of odorant were determined using GC analysis.

Results The n-butanol regression slope gave a high R^2 value (0.971) and low scatter. However, the data did not correspond to that of other workers using an ASTM method where the range of recommended butanol concentrations was insufficient at the high end to determine the top of the organoleptic scale and insufficient at the low end to determine threshold. Moreover, headspace analysis using GC confirmed the published gas concentrations to be in error by a factor of 10. It was also observed that high concentrations of odourants were irritant causing desensitization if used for prolonged periods.

Conclusion A previously described method had erroneous headspace calculations listed and n-butanol could not be recommended as a training odorant because of its irritancy.

P37

Differences in score and inter examiner variation between four organoleptic methods

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Objective Organoleptic measurements are important tools for measuring halitosis. Many different methods have been applied, but an internationally recognized standardized method has not yet been agreed on. We hypothesized that four various organoleptic methods: (A) the open mouth method, (B) the counting method, (C) the 'HA-HA-HA' method (determining breath while the subject says HA-HA-HA), and (D) the glass straw method, may produce different scores and inter examiner variations. Thus the aim of this study was to compare the scores and inter examiner variations obtained by these four methods.

Methods Five trained (UWE Bristol) odour judges (OJs) assessed the breath of 20 test persons (17–71 years) by the methods A to D (see above), in a randomised and blinded set up. The 0–5 organoleptic scale by Rosenberg *et al.* (1991) was used for determining the intensity of oral malodour. OJs were allowed to score in fractions of 0.25 score. Inter examiner variation was calculated as the average percentage deviation for the scores of each OJ (computed from the mean score of the five OJs).

Results The mean organoleptic score of the 20 subjects differed significantly depending on the method applied ($P < 0.001$). Method (A) produced a score of 1.20 ± 0.88, (B) 1.69 ± 0.83, (C) 1.80 ± 0.89, and (D) a score of 2.11 ± 0.92. Further a significant difference was obtained for the inter examiner variation between the four methods ($P < 0.001$). The inter examiner variation for method (A) was

55%, for (B) 30%, for (C) 31%, and for (D) 26%. Finally a significant correlation was obtained between the organoleptic score and the inter examiner variation ($r_s = -0.70$, $P < 0.001$) becoming lower with higher scores.

Conclusion Due to the higher mean scores and the lower inter examiner variation the glass straw method may be preferable for organoleptic research studies of halitosis.

P38

Transferred to oral session

P39

Evaluation of patient oriented outcome using SF-36 in halitosis therapy

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Objective To evaluate the patient oriented outcome of medical interventions, Medical Outcome Study Short Form-36 (SF-36) is a widely used, self-administered questionnaire. The SF-36 has eight dimensions of health status consisting of Physical Function (PF), Role- Physical (RP), Bodily Pain (BP), General Health (GH), Vitality (VT), SF (Social Function), Role-Emotion (RE) and Mental Health (MH). Furthermore, each dimensional score and two summary scores about physical and mental regions can be calculated by the algorithm. The aim of this study was to examine the application of SF-36 to the clinical parameter of halitosis.

Subjects and methods Seventy patients visiting our special clinic for halitosis were the subjects. In this study, we defined the healed persons as follows. They received our therapy two or more times, and the duration was over 2 months. After our therapy, they declared that the oral malodor of themselves could not bother them. In addition, they accepted to receive the general dental check-up regularly after the initial therapeutic period. The SF-36 was filled by the subjects at the first visit of our clinic and the first time of the regular check-up. The content of our therapy was mainly involved measurement of oral malodor, supporting for oral self-care, professional oral cleaning and counseling. All management concerning SF-36 was performed according to the manual of SF-36, version 1.2, Japanese edition.

Results and discussion Among all 70 subjects, GH, VT, SF, RE, MH and mental summary scores were significantly lower than national averages. Among the healed persons ($n = 17$), only SF score significantly rose after receiving our therapy. These results suggest that the consciousness of the improvement in social function could be an important outcome of halitosis therapy. In conclusion, measuring health related QOL using SF-36 might be useful for evaluating the efficacy of halitosis therapy.

P40

Measurement of VSC and organoleptic scores and subjective patients' opinion about halitosis – a questionnaire study

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Objectives The aim of the study was to evaluate the level of interest in fetor ex ore among respondents, patients of the Department of Conservative Dentistry in Warsaw.

Methods A questionnaire was completed by 295 patients, 202 female and 93 male aged 18–74 (avg 38.73). Each person was also examined for organoleptic score and VSC by halimeter. Halitosis was diagnosed if the average level of VSC measured by halimeter was ≥125 ppb and the organoleptic measurement using a 5-point scale was ≥2. Statistical analysis was performed using Chi square test.

Results Incidence of halitosis was greatest in age ranges 25–34 (29.68%) and 45–54 years (24.52%). People from these age groups are more careful about oral heath, so they are more likely to be interested in checking for halitosis. Among 295 respondents 25.42% often had a problem with bad breath, 39.66% sometimes, and 34.91% rarely or never. Persons with diagnosed halitosis frequently reported having a problem with bad breath (47.73%), and only 5.76% persons with halitosis did not notice this problem ($P < 0.001$). Among 76 healthy persons five (6.6%) reported having a problem with halitosis ($P < 0.001$). Clinical tests for halitosis allowed a diagnosis of halitophobia in four cases. Respondents noticed a problem with bad breath in their relatives in 46.0%. The frequency of malodor observed in relatives of patients with halitosis was similar to that in relatives of healthy subjects ($P = 0.58$). No correlation was found between cigarette smoking and a diagnosis of halitosis ($P = 0.22$).

Conclusions A statistically significant correlation was found between clinical diagnosis of halitosis and VSC level by halimeter, organoleptic measurement and subjective patients' opinion.

P41

Tongue cleaning habits in Italians: results of a survey in a sample population

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Background and objectives Mechanical cleaning of the tongue is suggested as an effective method for reducing cultivable flora of the tongue and teeth and decreasing oral malodor. The aim of this study was to evaluate the general attitude, knowledge and behaviour of a representative sample of Italian population regarding the hygienic tongue procedures.

Methods The survey has been conducted in January and February 2003 through structured phone interviews using CATI (computer aided telephone interviews) methods by EuriskoOmnibus, a specialized agency, on commission by GlaxoSmithKline Consumer Healthcare S.p.A.-Italy. The

sample has been randomly chosen among urban and rural residents from age 15 onwards with a nationwide distribution in proportion to the last demoskopic feature of general Italian population. Relevant data has been processed with MSExcel and StatView software in an Apple G4 Powerbook PC.
Results The sample is composed by 2000 individuals, 52% females and 48% males. Of these, 6% have an academic degree, 23% high school and 71% lower middle school Over one-third (39%) of Italians interviewed declared to perform specifically tongue cleaning: 40% of them every time they perform oral hygiene twofold or more daily, 24% once a day and 36% less than daily; the tongue cleaning habits are more diffuse in younger people. Seventy-one per cent of tongue cleaners perform the procedure with an ordinary toothbrush, 22% declared to clean tongue with an oral rinse and only 4% utilize specific tongue instruments. In our sample main reasons for tongue cleaning practices were reaching a better oral hygiene (60%) and fighting bad breath (25%).
Conclusions In Italians tongue cleaning is more diffuse than expected but the reasons are not principally related to the reduction of halitosis.

P42
Subjective symptoms and knowledge of oral malodor on dental students in Thailand

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The purpose of this study was to investigate subjective symptoms about oral malodor and the knowledge and attitude for bad breath on dental students. The subjects were 213 students from 1st to 6th year undergraduate course of one dental school in Thailand (male: 70, female: 143). Their mean (s.d.) age was 21.0 (1.9). Questionnaire survey was conducted in November 2003. Questionnaire items were subjective symptoms about oral malodor and dry mouth, the knowledge and attitude for bad breath, oral hygiene practice, smoking/drinking habits, and life stress. Five students (2.3%) answered that they concerned their oral malodor often and 87.8% reported sometimes. Of them, 72.9% wanted to receive treatment for their malodor and 70.8% said they suffer from bad breath in daily life. Two-thirds of students perceived strong oral malodor when waking up. There were no significant differences of the self-perceived malodor rates between gender or among year of study. More than half of the students did not know that oral diseases and poor oral hygiene are the major causes of bad breath. The students' attitudes for bad breath were different if the target was family members or friends. The majority of the students (88.7%) answered they perceived dry mouth. Perception of dry mouth was significantly related with self-reported oral malodor prevalence ($P < 0.05$). However other oral or behavioral factors did not relate to the self-reported malodor rates. In this study, dental students perceived bad breath in high prevalence. However the knowledge for bad breath was not enough even the students who already provided dental treatment to the patients. It would be necessary to give them adequate knowledge and methods in education program for managing not only patients' but also their own oral malodor problem.

P43
Effectiveness of a mechanic-chemical method in reducing malodor of dentures

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Objective of investigation To assess effectiveness of a mechanic-chemical denture cleansing to reduce malodor in denture wearers in comparison with a brushing only method.
Methods Twenty-nine denture wearers between 50 and 60 years old, 6 months after denture delivery were trained to clean their dentures with denture brush and soak in normal saline at nights for 1 month. The malodor of dentures was detected with a bad breath detector (VSC monitor: Tanita, Japan). In the following month the subjects were trained to clean with a denture brush but additionally soak in antiseptic mouthwash (Listerine, USA) each night and soak in 5% sodium hypochlorite twice a week for 5 min. At the end of 1 month the malodor of dentures was again assessed using the VSC monitor.
Results and discussion For denture brush alone all cases showed severe malodor VSC levels. Following the mechanic-chemical cleansing, 20 cases gave moderate and nine cases gave mild malodor VSC levels. Statistical analysis with Wilcoxon test determined the mechanic-chemical method to have been significantly effective (P-value < 0.05). The results show that for reducing malodor of dentures, denture brush alone is insufficient and use of antiseptic materials are necessary. Some studies have stated that allergic reactions to sodium hypochlorite are possible and in addition denture cleansing may be difficult for disabled or elderly patients. Therefore, future studies should focus on non-allergic disinfectants and/or simpler/easier methods (recommended protocols) to carry out effective disinfection.
Conclusion The mentioned mechanic-chemical method proved to be effective at reducing malodor of dentures whilst brushing alone was insufficient.

P44
Management of volatile sulphur compounds with ozone

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Volatile sulphur compounds (VSCs) are responsible for oral malodour (bad breath). There are many otc products for people for use, based on an oxidative reaction system. This paper examines the effect of ozone on VSCs.
Objective To test the efficacy of Ozone (O_3) dissolved in water on VSCs and the management of bad breath in a small pilot study in a general dental practice environment, test the Halimeter, and test a new bad breath Index, the Ks Index.

Method Twenty subjects were recruited into this study. After olfactory assessment, they were assigned to a Ks Index (The Kissability Index) and measured with the Halimeter (Interscan Corp, USA) after calibration, to establish their base-line VSCs. Each subject gargled with ozonated water for 30 s, and were re-assessed with the Halimeter. This was repeated after 5-, 30- and 60-min intervals. Ozone (O_3) was bubbled under pressure into 1 l of cooled, distilled water at a concentration of 2100 ppm for 1 min for each subject.

Results After just 1 min of gargling and mouth rinsing with 100 ml ozonated water, there was an average VSC reduction of 42.5% from the pretreatment baseline value. Five minutes after treatment, the VSCs rose an average of 14.2%; after 30 min, a further 18.9%, and after 60 min, the VSC values had risen by a further 24.6%. Ozone is a powerful oxidant, and in gaseous form, is used to reverse caries (1). When dissolved in water (maximum 5% volume in distilled, cooled and pressurised water) ozone is safe, and has been extensively used in reducing cfu's in dental water lines (2). Studies in Russia (3) have shown ozone to be an effective oral hygiene adjunct. This study showed that low concentrations of ozonized water is cheap and easy to make. It is a very effective product to reduce VSC's, and further research is proposed in this area.

Conclusion Ozone dissolved in water may offer a cheap alternative to the more expensive and less effective commercial mouth rinses to combat bad breath. However it is unstable (half-life of about 2–3 h if kept cold) and needs to be manufactured when required. It may have a greater role in the control of oral hygiene or as a presurgical mouth rinse.

P45

Microbiological culture analysis of the tongue microflora in subjects with and without halitosis

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Objective Determination of the microflora present on the tongue dorsum of subjects with and without halitosis using conventional microbiological culture methods.

Methods Twenty-one halitosis and 20 control patients were recruited using a strict clinical protocol. Samples were collected from the posterior dorsum of the tongue using a sterile brush. Each sample was vortex mixed for 30 s and serial 10-fold dilutions to 10^{-7} were carried out. Samples were plated onto fastidious anaerobe agar (FAA) and FAA enriched with vancomycin. These were incubated under anaerobic conditions for 10 days at 37°C. Strict anaerobes were identified by metronidazole sensitivity and bacteria were identified to genus level by a combination of colony morphology, Gram staining and biochemical and enzymatic tests (rapid ID 32 A).

Results The predominant species in test and control groups were *Veillonella* and *Prevotella*. Greater species diversity was found in the halitosis samples compared to controls. The halitosis samples contained a larger number of unidentifiable gram-negative rods, gram positive rods and gram negative coccobacilli.

Conclusions There was no obvious association between halitosis and any specific bacterial genus. The increased species diversity found in halitosis samples suggests that halitosis may be the result of complex interactions between several bacterial species. The role of uncultivable bacteria may also be important in contributing to this process.

P46

Stable storage of chlorine dioxide mouthrinse solutions

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Chlorine dioxide (ClO_2) is a highly effective yet non-toxic malodor counteractant and germicide that is listed as an ingredient in many mouthrinses. It is a water-soluble gas whose purported chemical instability and permeability through common storage containers has resulted in its inability to be provided as a shelf-stable, marketable solution of significant concentration. Thus ClO_2 has had to be generated *in situ*, or shortly before use, which has limited its utility. The object of the present investigation was to evaluate ClO_2 concentrations over time in aqueous, near neutral pH, ClO_2-ClO_2^- (1:1–1:14) solutions (32–40 ppm ClO_2) prepared according to the method of Richter in containers made from a variety of materials under ambient, accelerated storage and other conditions. [ClO_2] was measured with standard spectrophotometric technique. All containers tested were standard 16 oz. bottles. The materials tested were glass of various colors and opacities, polyvinyl chloride (PVC), high-density polyethylene (HDPE), fluorinated HDPE, high-density terephthalate (PETE) of various colors and fluoridated polyolefins. We discovered that certain materials, such as glass, fluoridated HDPE, clear and white PETE and fluorinated polyolefins can maintain relatively constant levels of ClO_2 in pre-prepared solutions and in some cases for periods of up to several years under ambient and normal shipping conditions. In one series of studies, the mean loss of ClO_2 per day from solutions prepared to approximately 35 ppm was 0.8 ppm (s.d. = 2.9) (2.2%) for HDPE (32 days); 0.8 ppm (s.d. = 2.2)(2.16%) for PVC (30 days); 0.4 ppm (s.d. = 0.71)(1.20%) for fluoridated HDPE (45 days); 0.02 ppm (s.d. = 0.63) (0.045%) for PETE (45 days) and 0.14 ppm gain (s.d. 0.54)(0.31%) for clear glass (31 days). The s.d.s reported here are skewed high because they assume a linear loss of ClO_2 over time when in fact the actual measured loss (or gain) was in all cases nearly perfectly parabolic. In another study, one supplier's white opaque PETE actually showed a 6.2% overall gain in 252 days of storage. These results dispel the popular notion that ClO_2 always decomposes so rapidly that fresh solutions must be made up frequently to maintain effective ClO_2 concentrations. ClO_2 production from the reservoir of ClO_2^- in these solutions is apparently sufficient to equal or exceed losses of ClO_2 from decomposition, diffusion through the container wall, possible reaction with the container material and escape during sampling for some of the materials tested. Previously observed losses of [ClO_2] from ClO_2-ClO_2^- solutions were apparently due primarily to diffusion out of the containers rather than

from decomposition within the containers. This achievement now allows for the broad distribution of pre-prepared ClO_2 solutions for a wide range of oral care and other deodorant and germicidal applications.

P47
Creation of longer-lasting (substantive) oral care flavours

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Mainstream oral care flavours are primarily designed to provide hedonic (taste) benefits and to promote breath freshness. In particular, a key criterion for a commercially successful toothpaste flavour is a long lasting reduction in the perception of mouth odour. Various additives can help deliver this benefit, in addition to flavours formulated according to patented guidelines. The residence time (substantivity) in the mouth of flavours and additives is clearly critical with respect to the longevity of the breath-freshening benefit, and little data are available in the literature to guide the selection of substantive components. The aim of this project was to investigate the dynamics of flavour loss from the buccal cavity following brushing using a mass spectrometer equipped with an atmospheric pressure headspace sampler which enabled real-time determination of flavour components in mouth air. A number of flavour ingredients found in peppermint- and spearmint-based oral care flavours were studied. The *in vivo* decay kinetics of flavour ingredient loss were quantified and found to be strongly related to the physicochemical properties of ingredients, except in the case of esters where a more complex dependence was observed arising from chemical transformation occurring in addition to physical transportation away from the mouth. Surprisingly, some materials were discovered to undergo rapid degradation with a half-life of minutes. Confirmatory studies of the decay kinetics of such materials were carried out *in vitro,* and structural features were identified which were associated with the observed hydrolytic vulnerability. This work has allowed the development of new guidelines to enable the creation of longer-lasting oral care flavours.

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Workshop reports

ISBOR Workshops on Oral Malodor and ADA Guidelines

Workshop 1
Odor measurements using human judges (including judge training and standardization)

Convener: Sushma Nachnami
University Health Resources Group Inc., UCLA, Canterbury Drive, Culver City, CA, USA

This group consisted of participants from various academic institutes and industry:

Most of the academic research groups had no formal training program in place. Several of the group members had worked with John Greenman's group to train and standardize. Also, a couple of academicians did set up some type of training where they at least worked together smelling human breath to train and calibrate before they began seeing patients. However the industry had elaborate training programs in place.

Samples used by industry for training were mostly garlic and onions; some of the groups used scratch & sniff tests or essential oils.

Organoleptic judges should have training documentation after completion of training. This should be recommended to the ADA.

Airborne infections: Concern about sniffing patients in close proximity was raised. Some people in industry monitor their judges closely by conducting medical exams on an ongoing basis. Medical histories of the patients should be looked at closely. Universal precaution laws should be followed closely.

How to sniff: Different methods were discussed, 'blinding' helps. Different ways of sniffing – some used glass tube, breathing out, e.g. counting out loud to twenty or saying ha ha ha etc.

Descriptors should be provided to the group for training as it is difficult for organoleptic judges to come up with appropriate phrases. While most of the academic groups trained directly with subjects, several academic groups reported developing training programs based on ASTM standards. The people from Industry all used ASTM standards. Certification was provided after 1 year of training with subjects.

Workshop 2
Odor measurements using instruments and other laboratory tests (GC, GCMS, Halimeter, Sensors, HPLC, OK2KISS, BANA etc)

Group convener: Professor Mel Rosenberg
Professor of Human Microbiology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

ADA guidelines stipulate that organoleptic/hedonic odour judges are the 'Gold Standard' whereas instrumental (e.g. gas chromatography) and microbiological analyses of oral malodour are considered secondary tests.

1. Why is instrumental measurement considered 2nd best to odour judges?

Comments of the participants included:

- Human judges are more commonly used.
  Gas chromatography (GC) is used to measure volatile sulphur compounds (VSCs), which are only part of the total breath mix.
- To what extent do VSCs contribute to breath odour? They correlate with odour scores but are not the only odour in breath.
- First, define other odorants in breath to produce simulated mixtures then compare these mixes to the real thing.
- There are probably different types of bad breath from different locations within the oral cavity.
- Methyl mercaptan is important, in determining the source of oral malodour.
- Butyric acid is odorous and present at high levels in saliva but does not play as important a role in breath odour.
- It is generally accepted that instrumental measures are not advanced or sophisticated enough to measure oral malodour.
- However, judges are subjective and there is a real need for 'equipment' that can be taken into the field.
- An inventory of the chemical constituents of bad breath must be completed because VSCs are not the only important component they are just the 'easy' route to measuring bad breath. Indole, skatole, butyric acid: Are they important?
- Use GC-MS and profile the breath of 1000 patients. This is not necessarily straightforward because it is possible to introduce false positive results by concentrating breath samples.
- Often, breath odour treatments can reduce the concentration of VSCs measured in a patients breath but the patient still has oral odour suggesting that other components are important.
- VSC levels in breath can be elevated by cysteine challenges, what components in breath are elevated after challenging with other amino acids?
- Microbiological techniques show increased numbers of *Streptococcus salivarius* in patients without oral odour, could this be the emergence of the 'good guy'?
- There have been no long term microbiological studies looking at ecology shifts in patients with and without oral malodour.
- Would long term microbiological studies provide useful information given that many species in the oral cavity are non-culturable and many are unknown?
- J. Tonzetich examined different components of oral malodour before he focused on the GC measurement of VSCs.
- The Halimeter® has been demonstrated to have a correlation of about 0.5 with odour scores.
- BANA (benzoyl-DL-arginine-napthyamide) and other enzymatic test have been shown to correlate with odour scores.
- Correlations between odour judges and other techniques for assessing oral malodour do vary and this depends on the number of odour judges used.
- Quest International has designed a screening system to find flavour components that specifically eliminate VSCs.

Reducing VSCs results in reduced odour in most subjects but there will always be exceptions.

- There are still studies that show the opposite effects for VSC and odour scores.
- Concentrated whole breath should be assessed – although alkanes have been shown to be present in oral odour, the odour index of alkanes is low.
- Do researchers actually look for other molecules in oral odour, other than VSCs?
- Colgate measure VSCs but are moving into the measurement of volatile fatty acids, indoles and amines using SPME.
- GC is a good predictor for the activity of potential odour reducing products. The hierarchical testing procedure for new products is usually as follows: *in vitro* to Halimeter to GC to organoleptic/hedonic odour assessments.
- What is the cost of using odour judges? Studies using odour judges are expensive, certainly more costly that using instrumental measures, and this could cause problems for smaller companies who do not have sufficient funding.

2. Will there be a day when an analytical measure is the 'gold standard' for assessing oral odour?

- Can we produce bad breath in a bottle?
- There is a difference between chronic and morning breath. The industry is geared towards treatment of morning breath. Do such treatments work for sufferers of chronic bad breath?
- There is no information correlating morning breath with mid-afternoon breath.
- How important will bacterial tests become? Have bacterial tests such as BANA been used in the professional field?
- Industry is driving the use of the Halimeter®. Maybe the BANA test should be marketed similarly to the Halimeter®.
- The BANA test needs money and marketing, as does the OK to KISS test.
- Is it possible to test odour treatments on bacteria, where the measurable end product is gaseous headspace? Caution must be exercised since bacterial metabolism will be effected by culturing conditions.
- Some microorganisms have been associated with oral odour, such as *Porphyromonas gingivalis*, *Prevotella intermedius*, however the problem is that a large percentage of the oral flora is non-culturable.
- What is the importance of postnasal drip in oral odour?
- How does tongue cleaning effect oral odour?
- Can amino acid mapping in the oral cavity be correlated with oral odour? (e.g. measuring the concentrations of methionine, cysteine, tryptophan at different locations in the mouth).

3. Will there ever be a 'gizmo' to measure oral odour with a 0.9 correlation with odour judges?

- Is this the promise of the electronic nose? Current problems with the electronic nose include moisture and must choose the right sensors. A researcher from University of Manchester Institute of Technology (UMIST) working with the electronic nose forecast that the technology would be ready by 2008!

General concensus

- Odour judges 'measure' whole breath odour, which is why they are considered the gold standard measure of oral odour. However research has shown that the human nose exhibits a Log response to stimuli but the scales used to rate oral odour are linear and that there is no pan-industry standard for this measurement, in terms of calibration of judges.
- VSCs are important components of oral odour and GC is the most accurate method of measuring VSCs in breath. Therefore the GC is the 'gold standard' for measuring VSCs.
- Portable VSC measures include the Halimeter and portable GC (requires validation). Other measures include enzymatic tests and microbiological measures. Performing all these measures in combination should give better correlations with odour judge scores.
- Research should be performed in the following areas:
  Detecting the component gases in oral odour.
  Defining 'bad breath in a bottle' and olfactory thresholds.
  Measuring oral odour substrates/substrate availability.

Suggestion for next ISBOR meeting Gather odour judges from research and industry and have them assess a cross-section of oral odour patients, in parallel with the instrumental measurement of odour.

Workshop 3
Role of microbiology (ecological shifts, agent resistance and efficacy)

Group convener: John Greenman
Centre for Research in Medicine, Faculty of Applied Sciences, UWE, Bristol, UK

The convener introduced the ADA sections that were relevant to microbiology and noted (in guideline section headed 'Scope') that oral malodor can be measured in a number of ways including '...bacterial enzymes'. The group noted that Microbiology was specifically mentioned in connection with two main areas, safety and efficacy (see sections 1 and 2 below) but was absent with regard to monitoring etiological agents themselves or proportional microbial markers or indicators of these agents (such as enzymes) (section 3 below).

1. Safety: Microbiology was specifically required (see ADA guideline section 7E and page 10) to show that potentially damaging shifts in oral flora populations (e.g. *S. aureus*, *Pseudomonas*, *E. coli*, yeast etc) should not occur during a 6-month human study. However, we might expect that a chemical agent if it was working properly would change the ecology to some extent (e.g. an oxidant might target anaerobes) and if it did not, was probably not working.

The group noted that an ecological shift of some type is inevitable when a complex microbial community is exposed to treatment. The subsequent findings would depend on which groups of microbes are searched for or detected, or even which methods are being used (including clone-specific molecular methods). The group thought it would be better for the ADA to discriminate between desirable shifts (e.g. one with fewer strict

anaerobes or VC producing types) and undesirable ones (e.g. with opportunistic pathogens as listed above). Another point was to consider that the baseline flora of individuals with malodor was already skewed in the direction 'disease phenotype' for malodor production, compared to one with fewer anaerobes. How do we define what we mean by a healthy or normal population? It was pointed out that ideas about normal/abnormal ecological balance are widely available in current probiotic literature. The limitations of an approach based on cultivation (e.g. on selective media) compared to modern molecular methods (probes or DGGE) was reiterated and it was hoped that the ADA would be aware of rapid changes in understanding and technology arising from these fields when updating or revising guidelines for the future.

2. Efficacy: According to the guidelines, development of resistance should be monitored at 0, 3 weeks and 6 months (of continuous treatment regime) in a representative sample of oral malodor micro-organisms (see ADA guideline sections 8E and page 11). The group noted that the ADA had not suggested which species should be monitored nor which samples should be used for purposes of monitoring. Should single isolates be picked and tested individually (for change in MIC or MBC) or should the mixed sample be plated as a whole onto plates or broths with test agents? More protocol guidance could have been given regarding the microbiological techniques, methods and standards.

The group noted that resistance of complex microbial communities as a whole is much less explored than those of pure species. Many thought that it was inevitable that some type of ecological shift would occur when microcosms are exposed to agents and that non-detection would simply reflect the lack of sensitivity of test methods used. The group thought that it was important to differentiate selection/adaptation changes (a natural feature of a microcosm following exposure and giving moderately increased resistance) in comparison to big resistance changes due to true genetic mutation or plasmid exchange followed by selection. Moreover, changes in resistance or ecology (if detected) following a 6-month trial should be monitored for a further period to see when (or if) they return to original baseline levels when treatment was stopped (this continued monitoring was required by ADA for anti-gingivitis but not for anti-odor agents). It was remarked that the biofilm literature was very extensive in the 'resistance' area.

3. Microbes as etiological agents: A third area for microbiology that was not emphasized in ADA guidelines would be in determining the causal agents of VC production (or a suitable marker or indicator for these agents such as microbial enzymes). If it is accepted that quantity of agents/enzymes is the key determinant of VC generation in malodor then it seems natural to measure this quantity and relate it to efficacy. This could be done in many ways since total load, total anaerobes, Gram-negative anaerobes and sulphide producing colonies should all be proportional to etiological agents in question. It was noted by the group that some agents could work by inhibiting enzymes or by neutralizing odor (e.g. new encapsulated adsorbents) and that this might not affect underlying numbers of microorganisms. However, for agents whose mechanism of action was through killing or otherwise reducing numbers of microbes, then evidence of reduction (by microbial monitoring or via new quantitative measures based on molecular biology) would constitute good evidence of efficacy, as powerful as those of breath measures. This would be especially pertinent if companies were claiming that their product was responsible for reducing/killing the microbes responsible for malodor. Others in the group pointed out that microbiology of this sort would be presented under section 8D (Mechanisms of action) of the ADA guidelines. Others thought it would be a valid measure on its own and should have been given more prominence in the guidelines.

Other points raised in the workshop centered on:

1. Type 1 (chemical) *vs* type 2 (mechanical) systems: Why should a type-2 gadget require 6-month trials for population shifts, when a toothbrush (which can be used equally well on tongue) can escape this type of testing! If it is a requirement to demonstrate that gadget usage does not predispose to ecological shifts, then the same requirement should apply to brushes or other implements used in oral cavity for oral care.
2. From the gingivitis, periodontal and caries literature it is clear that some tests could be used to prevalidate some of the products/agents likely to be tested (e.g. *in vitro* tests). There is considerable experience already in these areas.

Workshop 4
Breath odor trial design and statistics

Group convener: David Bradshaw
Quest International, Ashford, Kent, UK

About 25 attendees participated in this workshop. There was a wide range of views expressed – and as other convenors mentioned – more questions raised than answers found. Perhaps some of these could serve as future topics for discussion at ISBOR conferences. Any errors or omissions reflect entirely on the convenor – please do feel free to suggest additions or amendments as appropriate.

Preselection of panelists & the ADA approval guidelines There was much discussion on the question of preselecting panelists (subjects) on the basis of malodour score. The ADA Acceptance Program Guidelines indeed requires 'the population should be at least 2 ± 0.5(mild malodor) on an intensity scale of 0–5, or a similar level on other appropriate scales'. Thus for compliance with the guidelines, some degree of screening is probably necessary. Some suggested that 'a product to solve a problem, needs to have a problem to solve'; others preferred the 'a product for everyman, everyday' approach. Furthermore, the ADA guidelines also require 'In the clinical evaluations, 80% of the subjects shall demonstrate a reduction to a 0 or 1 rating based on a 0–5 organoleptic intensity rating...' Since companies will be keen to gain ADA Acceptance, there is the possibility that study designs could be biased toward recruiting panellists with a minimum acceptable score (2 ± 0.5), with a view to complying with the reduction to 1 or below, post-treatment. For example, recruitment of a panellist with a resting score of 3 would increase the risk of a product failing to gain acceptance.

Some concern was expressed that since the ADA guidelines simply state 'Depending on the claims being made, oral

malodor measurements should be performed at a minimum of two appropriate time periods after baseline during the three-week test period' that potentially almost any product could work – if very short time intervals were selected.

Some participants suggested that such concerns could be addressed by 'levelling the playing field' – even if this was achieved 'artificially' – for example by cysteine rinses etc. Some commented that sulphur compounds do not represent 'all' of oral malodour; others commented that *only* VSC measurements consistently correlated with organoleptically-perceived malodour. This then is a question that clearly calls for further debate.

General statistical issues A *crossover* trial design – where each individual acts as their own control, reduces the possibility of inter-individual variations influencing study outcome (as could be the case in a parallel design). As it is well known in oral malodour research (as well as in most other such clinical studies), that *inter*-subject variation is much greater than *intra*-subject variation, a crossover study design is *inherently* more powerful than a parallel design for the same number of subject × sample combinations. Crossover designs do, however, require an appropriate washout period (see below).

Pretrial procedures What should be the oral hygiene procedures preceding panellists taking part in a trial? How long a gap should there be prior to trials since the last oral hygiene routines? What restrictions on diet and other oral-related habits should be imposed? Someone suggested that screening should establish a baseline malodour score over time. Are there known circadian rhythms in malodour?

Breath odour scoring on the 0–5 scale Noted that a score of 0, or of 5 is extremely rare ($< 0.5\%$ of the population). Likewise, scores of 4 are probably $< 5\%$. So for the most part the 0–5 scale is actually a 3-point scale – 1, 2 or 3. Some workers have thus used ½-point scales, as a useful and pragmatic solution to the conflicting objectives of encompassing a full odour intensity range in a 0–5 scale, whilst maintaining sufficient sensitivity to distinguish between samples. Other workers stick to the integer-scale. The issues around statistical treatment of the data were discussed. Some suggested that a 'pseudo-continuous' ½-point scale would allow statistical methods such as ANOVA to come into play. Others preferred to stick to medians, and then to use non-parametric statistical methods. There is an entire literature on sensory perceptions of odour/malodour – maybe this should be something that ISBOR and other interested societies could pursue further.

Appropriate washout periods Participants debated the requirement for appropriate washout periods (and products) to be used in efficacy trials, i.e., to standardize usage of other oral care products between use of products within a trial. However, the appropriate washout period for products would very much depend on their efficacy and/or their mode of action. For a regular oral care product such as a toothpaste, 1 week might be an appropriate timescale; a strong antimicrobial product might require a longer washout period. In the case of probiotics, where the potential for activity might stretch almost indefinitely – what washout period would be appropriate? Some of these issues can be addressed by various statistical methods – the study design can ensure that all products are tested equally often in each relevant time period (to avoid issues such as changes in weather, for example). This is often achieved via a Latin-Square type design. Carry-over effects can be balanced by use of a Williams Latin square design, which balances the carryover between successive products by ensuring every product follows every other product with equal frequency.

Panellist numbers Some participants wanted to know the numbers of panellists that would be appropriate for trials. This clearly would depend on a number of factors, especially the statistical design of the trial. Other factors which would be important include (i) the relative efficacy of the products tested (compared with controls), (ii) the initial malodour scores of panellists, (iii) the magnitude of any differences which the study aims to detect. Other participants reported that their typical trials have used 10–15, or 20–30 panellists.

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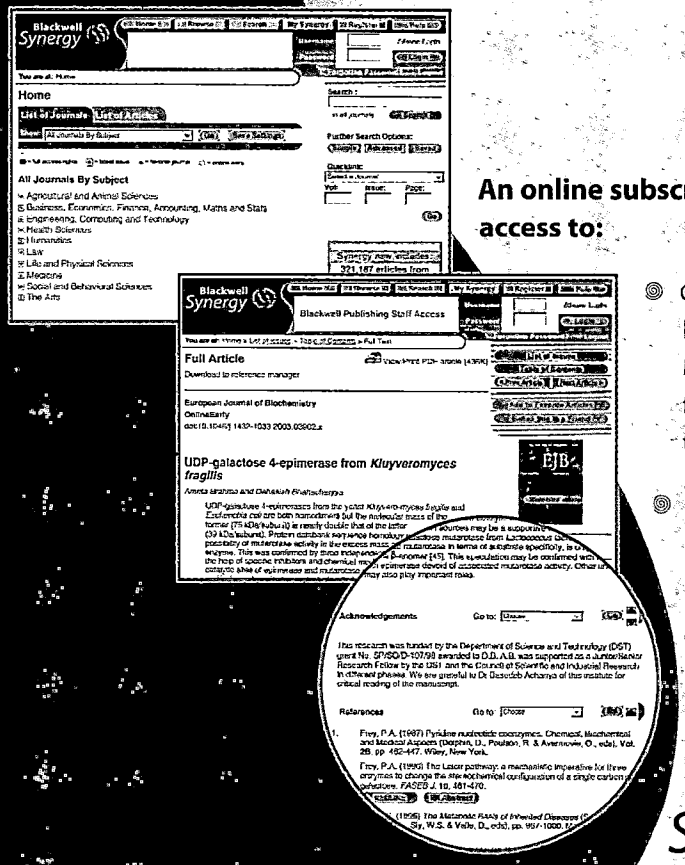

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Oral Diseases

INSTRUCTIONS TO CONTRIBUTORS

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6 Discussion This should comment critically on the probity of the results obtained, their relationship to existing knowledge and significance for improved understanding of oral diseases. Speculation and new hypotheses are encouraged, provided they are firmly rooted in the data presented. Conclusions should be drawn.

7 Acknowledgements

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Complete book:

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Chapter in book:

Moller IJ (1992). Endemic dental fluorosis. In: Prabhu SR, Wilson DF, Daftary DK, Johnson NW, eds. *Oral diseases in the tropics*. Oxford Medical Publication: Oxford, pp. 68–77.

Agency publication:

Gart JJ, Krewski D, Lee PN *et al* (1986). *Statistical methods in cancer research. Volume III. The design and analysis of long-term animal experiments*. International Agency for Research on Cancer: Lyon. IARC Scientific Publication No. 79.

Dissertation or thesis:

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